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PATHOLOGY

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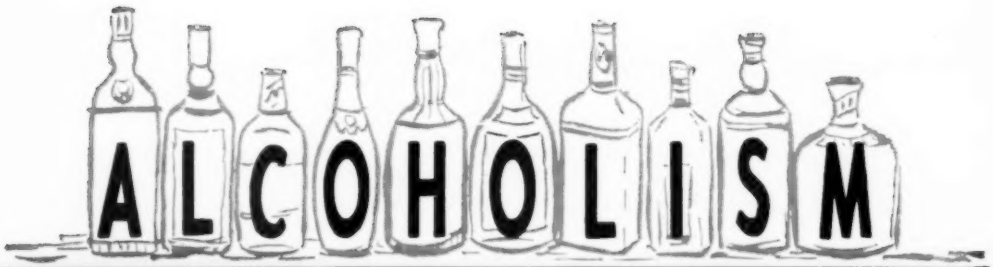

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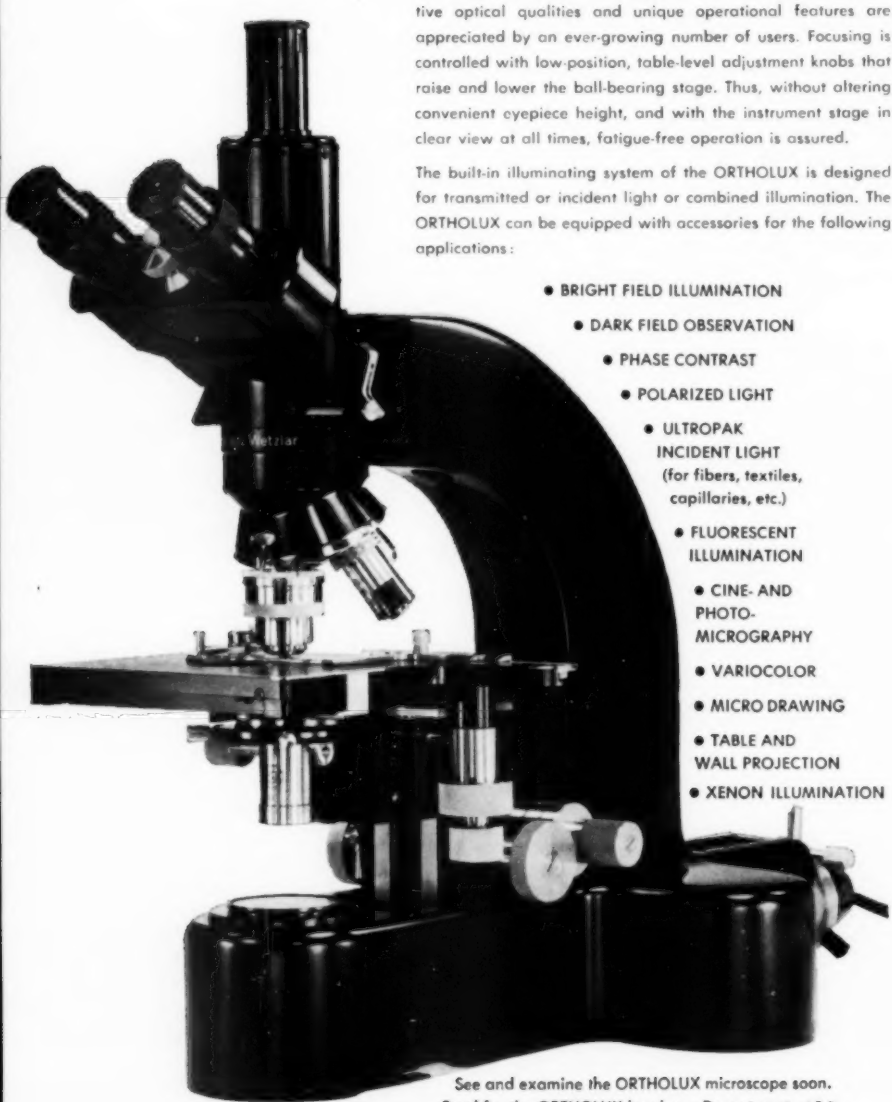

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PATHOLOGY

A Comparative Study of Four Groups of Acid-Fast Organisms, with Special Reference to Pathogenicity

WILEY D. FORBUS, M.D.; JOHN T. CUTTINO, M.D.; ALBERT G. SMITH, M.D.; ANNE McCABE MARGOLIS, B.S., and DORIS WINGATE REID, B.S., M.A., Durham, N. C.

In 1949 Cuttino and McCabe reported from our laboratory an extraordinary fatal granulomatous disease in a child which they showed to be the result of infection by an entirely new species of acid-fast organism.[1] To this organism they gave the name Nocardia intracellularis because of the almost constant intracellular position which it occupied in the tissues. The cytologic hosts were the cells of the reticuloendothelial system. These cells exhibited to an astonishing degree two of the six basic reactions of reticuloendothelial cells to stimulating agents, namely, phagocytosis and proliferation.[2] The other four basic reactions of these cells observed so commonly in other etiologic forms of genuine granulomatous inflammation, namely, motility, antibody formation, necrosis, and change in form and function, were almost inapparent. Because of this, the lesion, which we have characterized by the term pure granuloma, was so much like a tumor that the true nature of the pathological process was not recognized until the acid-fast organisms responsible were demonstrated in huge quantity in the

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Present address of Dr. John T. Cuttino: Dean, Medical College of South Carolina, Charleston, S. C.

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From the Departments of Pathology, The Duke University School of Medicine, Durham, N. C., and the Medical College of South Carolina, Charleston, S. C.

TABLE 1.—*Lesions Produced in Animals by the Nocardiae* *

		Mice		Rats		Guinea Pigs	
No.	Source & Source No.	L	S	L	S	L	S
1	Waksman 3520	4G	4	0	0	0	0
2	Waksman 3403	3G	1	0	0	0	0
3	Waksman 3407 N. erythropolis	4G	3	0	0	3	0
6	Waksman 3402 Nocardia	0	0	0	0	0	0
7	Waksman 3405 Nocardia	3G	3	1G	1	0	0
8	Waksman 3300 N. gypsoides	1G	0	1G	1	0	0
9	Waksman 3500 Nocardia	3G	2	0	0	1G	1
10	Waksman 3318 N. farcinica	1G	1	0	0	1G	0
11	Waksman 3306 N. asteroides (Bering)	1G	1	3	2	0	0
12	Waksman 3383 Nocardia sp.	0	0	0	0	0	0
13	Waksman 3471 Nocardia	1G	0	0	0	0	0
14	Waksman 3488 N. mexicanus	2G	0	0	0	0	0
15	Waksman 3376 N. maculatus (Millard-Burr)	0	0	0	0	1M	0
16	Waksman 3406 N. (serratis) corallina	0	0	0	0	0	0
17	Waksman 3399 N. farcinica	0	0	0	0	0	0
18	Waksman 3487 N. mexicanus	1	0	2	0	1	1
19	Waksman 3449 N. gardneri	0	0	0	0	0	0
20	Waksman (pathogenic N. asteroides Eppinger)	0	0	0	0	0	0
21	Waksman 3409 A N. polychromogenes	2G	0	0	0	0	0
22	Waksman 3410 N. paraffinae	0	0	1	0	2M	0
23	Waksman 3485 N. mexicanus	0	0	1	0	0	0
		—	—	—	—	—	—
Total organisms 21							
Total infected animals		26	15	9	4	9	2
Total animals given inoculations		84		84		84	

* L indicates local; S, systemic; G, granulomatous; M, mixed; A, acute suppurative.

TABLE 2.—*Lesions Produced in Animals by the Soil Saprophytes* *

No.	Source & Source No.	Mice L	Mice S	Rats L	Rats S	Guinea Pigs L	Guinea Pigs S
25	Hagan 7	3G	0	1G	0	3G	0
26	Hagan 35	1G	1	2G	1	0	0
27	Hagan 5	1M	0	1G	0	3G	0
28	Hagan 56	4G	0	2G	0	3G	2
29	Hagan 45	0	0	1G	0	2G	0
30	Hagan 10	2M	0	0	0	2G	0
31	Hagan 4	0	0	2G	0	3M	0
32	Hagan 54	3G	0	2G	0	3G	2
33	Hagan 55D	1M	0	2G	1	4G	0
34	Hagan 50	2G	0	1G	0	3G	0
35	Hagan 34	3G	0	3G	0	0	0
36	Hagan 70	2G	0	1G	0	3G	0
37	Hagan 42	2G	0	3G	0	0	0
38	Hagan 66	3G	0	1G	0	2M	0
39	Hagan 29	2G	0	0	0	1G	0
40	Hagan 69	3M	0	0	0	1M	0
41	Hagan 40	1G	0	0	0	1G	1
42	Hagan 51	3MG	0	2G	0	0	0
43	Hagan 39	2G	0	0	0	4G	2
44	Hagan 64	0	0	2G	0	2M	0
45	Hagan 116	3M	0	2G	0	2G	0
47	Hagan 122	0	0	1G	0	0	0
48	Hagan 111	1A	0	2G	0	1M	0
49	Hagan 114	1A	0	2G	0	2G	0
50	Hagan 115	2G	0	2G	0	2G	2
51	Hagan 82	1G	0	2G	0	0	0
56	Hagan 123	3G	0	2G	0	0	0
57	Hagan 121	0	0	0	0	0	0
59	Hagan 125	2G	0	1G	0	0	0
60	Hagan 126	3M	0	1G	0	0	0
61	Hagan 90	2G	1	0	0	1G	0
62	Hagan 83	2G	0	3M	0	0	0
63	Hagan 120	2M	0	2M	0	1G	1
64	Hagan 73	1A	0	1G	0	0	0
65	Hagan 1036	2G	0	0	0	1M	0
67	Hagan 925 misc.	3A	0	2G	0	4M	0
68	Hagan 839 misc.	0	0	1G	0	4M	2
70	Hagan 129 lep.	2G	0	2G	0	0	0
71	Hagan 1943	4M	0	2G	0	1	0
72	Hagan 672 lep.	1G	0	3M	0	2G	0
73	Hagan 1652	1G	0	0	0	0	0
74	Hagan 5617	2M	0	1M	0	2G	0
75	Hagan 2563	0	0	0	0	1G	1
76	Hagan 909	1M	0	0	0	0	0
77	Hagan 844	2M	0	2G	0	2G	0
78	Hagan breed, K. B. N. C.	4M	0	1A	0	2M	0
79	Hagan 1023	2M	1	0	0	3G	0
80	Hagan 319	2G	0	0	0	0	0
81	Hagan lep. N. M. 2	1M	0	2G	0	3G	0
82	Hagan breed K. N. R. H.	0	0	1G	0	0	0
83	Hagan 843 MD	1G	0	2G	0	4G	0
84	Hagan 682 lep.	3G	0	3G	0	1G	0
85	Hagan 2562 breed	1G	0	0	0	0	0
86	Hagan 9452 misc.	3A	0	4G	1	2G	1
87	Hagan lep. MD	3G	0	2G	0	0	0
88	Hagan 9453 misc.	3A	0	2G	0	1G	0
89	Hagan R-74	3A	0	2G	0	4A	0
90	Hagan R-58	2M	0	3G	0	1G	0
91	Hagan R-91	2M	0	2G	0	0	0
92	Hagan R-50	3G	1	3G	0	0	0
93	Hagan R-71	4M	0	0	0	0	0
94	Hagan R-60	3M	0	2G	0	0	0
95	Hagan R-84	3M	0	4G	1	1G	0
96	Hagan R-83	4M	0	3G	0	1G	0
97	Hagan R-85	0	0	0	0	0	0
99	Hagan R-44	2A	0	2M	0	1G	0
100	Hagan R-79	2M	0	4G	0	1M	0
101	Hagan R-64	3A	0	2M	0	2M	0
102	Hagan R-86	0	0	0	0	0	0
103	Hagan R-87	2M	0	0	0	1G	0
104	Hagan R-76	1A	0	0	0	1A	0
106	Hagan R-62	2M	0	2M	0	4M	1
107	Hagan R-88	2M	0	2M	0	3A	0
108	Hagan R-100	1A	0	2M	0	4M	0
109	Hagan R-124	3A	0	3M	0	3A	0
110	Hagan R-120	1A	0	1G	0	0	0
111	Hagan R-125	3A	0	3M	0	2A	0
115	Hagan R-97	3M	0	1A	0	3M	0
116	Hagan R-123	3M	0	3M	0	4M	0
120	Hagan R-115	3A	0	0	0	3M	0
121	Hagan R-98	4A	1	4A	0	4A	0
122	Hagan R-113	1A	0	1G	0	2A	0
124	Hagan R-107	2A	0	0	0	2M	0
125	Hagan R-122	3A	0	1M	0	2A	0
127	Hagan R-68	2A	0	3M	0	3A	0
129	Hagan R-65	3A	0	4M	0	3M	0
130	Hagan R-78	2A	0	3M	0	3A	0
131	Hagan R-121	4A	0	2M	0	2A	0
132	Hagan R-117	4A	0	3M	0	4A	0
133	Hagan R-102	3A	0	1M	0	4M	0
135	Hagan R-93	4A	0	3M	0	3A	0
136	Hagan 5811 breed	0	0	0	0	4M	0
137	Hagan R-H misc.	1A	0	1G	0	1A	0
138	Hagan 550 misc.	3A	0	1G	0	4A	0
139	Hagan 773-H breed	1M	0	1M	0	0	0
140	Hagan 2662 breed	1M	0	0	0	1G	0
141	Hagan 902 misc.	0	0	2M	0	0	0
142	Hagan 2564, breed	1M	0	0	0	0	0
143	Hagan 171 misc.	0	0	0	0	3M	0
145	Hagan 51 R misc.	3M	0	4M	0	3M	0
146	Hagan R-109	2M	0	3M	0	2M	0
147	Hagan R-110	2M	0	2M	0	2A	0
148	Hagan R-94	2M	0	0	0	0	0
149	Hagan R-106	2M	0	4M	0	3A	0
150	Hagan R-23	4M	0	1M	0	3A	0
151	Hagan R-92	2M	0	2M	0	3M	0
152	Hagan 1076 lep.	3M	0	3M	0	2M	0
153	Hagan 110 lep-Nov. 1947	1M	0	1M	0	2M	0
154	Hagan breed K. B. N. K.	1M	0	0	0	1M	0
155	Hagan 113 lep.	1M	0	0	0	0	0
156	Hagan 903 misc.	0	0	2M	0	4M	0
158	Hagan 9454 misc.	4M	0	1M	0	4M	0
159	Hagan R-56	0	0	0	0	3M	0
160	Hagan R-21	4M	1	1M	0	3M	0
161	Hagan R-43	2M	1	1M	0	3M	0
162	Hagan R-45	3M	0	4M	0	4M	0
163	Hagan R-46	2M	0	4M	0	1A	0
165	Hagan R-57	0	0	1M	0	1M	0
167	Hagan R-37	1M	0	1M	0	2M	0
168	Hagan R-19	3M	0	2M	0	2M	0
169	Hagan R-59	1G	0	2M	0	2A	0
170	Hagan R-10	3M	1	1M	0	4M	0
171	Hagan R-55	1M	0	1M	0	3A	1
172	Hagan R-48	2M	0	0	0	1A	0
174	Hagan R-24	0	0	0	0	2A	0
175	Hagan R-3	2M	0	2M	0	4M	1
176	Hagan R-49	0	0	0	0	2A	0
178	Hagan R-52	1M	1	0	0	2M	0
179	Hagan R-1	4M	0	2M	0	4A	0
180	Hagan R-14	3M	3	1M	0	3M	0
181	Hagan R-35	0	0	1M	0	3A	0
183	Hagan R-18	2M	0	2M	0	3M	0
184	Hagan R-16	0	0	0	0	0	0
185	Hagan R-7	0	0	1M	0	3A	0
186	Hagan R-12	2A	0	2M	0	3M	0
187	Hagan R-27	1M	0	0	0	0	0
188	Hagan R-39	3M	1	1M	0	1M	0
189	Hagan R-15	2M	0	0	0	1A	0
191	Hagan R-33	2M	0	1M	0	1M	0
192	Hagan R-17	1M	0	1M	0	2M	0
193	Hagan R-41	2M	0	1M	0	1M	0
195	Hagan R-42	3M	0	1M	0	2M	0
196	Hagan R-32	2M	0	1A	0	1M	0
197	Hagan R-25	3M	0	4M	0	3M	0
200	Hagan R-8	0	0	2M	0	3M	0
201	Hagan 99	0	0	1M	0	1A	0
202	Hagan 96	0	0	1M	0	0	0
203	Hagan 91	0	0	0	0	0	0
206	Hagan 86	0	0	0	0	1A	0
208	Hagan 110	0	0	0	0	3A	0
209	Hagan 105	0	0	0	0	0	0
210	Hagan 103	0	0	0	0	0	0
211	Hagan 145	0	0	0	0	1A	0
212	Hagan 23	0	0	0	0	2M	0
213	Hagan 113	1M	0	0	0	0	0
214	Hagan 17	0	0	3M	0	1M	0
216	Hagan 80	0	0	0	0	1M	0
217	Hagan 1	0	0	1M	0	0	0
218	Hagan 127	0	0	0	0	0	0
219	Hagan 117	2M	0	1G	0	1A	0
220	Hagan 128	0	0	1M	0	0	0
221	Hagan 143	0	0	0	0	2A	0
222	Hagan 129	0	0	0	0	0	0
223	Hagan 148	0	0	0	0	0	0
	Total organisms 164						
	Total animals infected	281	13	223	4	281	18
	Total animals given inoculations	656		656		656	

* L indicates local; S, systemic; G, granulomatous; M, mixed; A, acute suppurative.

TABLE 3.—*Lesions Produced in Animals by the Mycobacteria* *

No.	Source & Source No.	Mice L	Mice S	Rats L	Rats S	Guinea Pigs L	Guinea Pigs S
316	Steenken H37 R (12T48) M. tuberculosis (human)	1G	0	0	0	0	0
317	D. K. Miller Meyer, Mem. Hosp., Buffalo Cicacelli—acid-fast organism; M. sp. unidentified.	2G	0	2G	0	2M	0
318	Aronson, H. Phipps Inst. N. berolinense	3M	0	0	0	0	0
319	Aronson, H. Phipps Inst. M. marpman	1G	0	1G	0	1G	0
320	Aronson, H. Phipps Inst. M. ranae	1G	0	0	0	1G	0
322	Aronson, H. Phipps Inst. M. avium No. 4	1G	0	1G	0	2G	0
325	Aronson, H. Phipps Inst. M. leprae (Clegg)	2G	0	2G	0	1G	0
326	Aronson, H. Phipps Inst. M. laeticola	0	0	0	0	1G	0
327	Aronson, H. Phipps Inst. M. leprae (Kedronski)	0	0	1M	0	0	0
328	Aronson, H. Phipps Inst. M. mist	2M	0	1G	0	0	0
329	Aronson, H. Phipps Inst. M. smegmatis (101)	0	0	2A	0	2G	0
330	Aronson, H. Phipps Inst. M. leprae (Duval, N. C.)	1G	0	0	0	2G	0
331	Aronson, H. Phipps Inst. M. chelonei	2G	0	3G	1	2G	0
332	Aronson, H. Phipps Inst. M. phlei (HPI)	1G	0	3G	0	1G	0
333	Aronson, H. Phipps Inst. M. butyricum (Rakins witch)	0	0	1G	0	0	0
334	Aronson, H. Phipps Inst. M. paratuberculosis	2G	0	1M	0	0	0
336	Acid-fast organism (tubercle bacillus ?) Kecoughton, Va. M. sp. unidentified	0	0	0	0	0	0
337	Santa Rosa strain (Santa Rosa Hosp., Texas) M. sp. unidentified	0	0	0	0	0	0
338	Acid-fast organism, Sidney Leopold, Baker V. A. Center Martinsburg, W. Va. M. sp. unidentified	3G	0	1G	0	0	0
340	M 9254 F. W. Wiglesworth, Children's Mem. Hosp., Montreal M. sp. unidentified	3G	0	2G	1	1G	0
Total organisms 20		—	—	—	—	—	—
Total animals infected		25	0	21	2	16	0
Total animals given inoculations		80		80		80	

* L indicates local; S, systemic; G, granulomatous; M, mixed; A, acute suppurative.

lesions, characteristically situated within the proliferating cells.

The above-noted limited response of the specific host, the reticuloendothelial cell, to this new acid-fast organism and the consequent (in a certain sense benign) form of host-parasite relationship, although not unfamiliar to us, called for a thorough study of the pathogenicity of other uncommon acid-fast organisms. Comprehensive studies of N. intracellularis are reported in Cuttino and McCabe's paper. In the present report are presented observations on (1) a group of 21 acid-fast organisms belonging to the genus Nocardia and representing 11 species (1 unidentified, Table 1); (2) a group of 164 acid-fast organisms of heterogeneous nature obtained from the soil and classified simply as soil saprophytes (Table 2); (3) a group of 20 acid-fast organisms belonging to the genus Mycobacterium (1 unidentified, Table 3); (4) a group of 3 acid-fast organisms belonging to the genus Actinomyces (Table 4).

In pursuing this study we have had certain specific objectives, namely, (1) to discover other strains of N. intracellularis and perhaps new species of pathogenic acid-fast organisms (The natural habitat of the organism recovered in the case reported by Cuttino and McCabe has not been determined. The patient was a native of North Carolina. As far as we know, N. intracellularis has not been recovered from any case other than that of Cuttino and McCabe. However, we have seen the typical lesion with organism morphologically identical with N. intracellularis in a single lymph node removed by biopsy from an otherwise unstudied patient in Canada.); (2) to gain

TABLE 4.—*Lesions Produced in Animals by the Actinomycetes* *

No.	Source & Source No.	Mice L	Mice S	Rats L	Rats S	Guinea Pigs L	Guinea Pigs S
312	Conant 1017, chest fluid, army private Actinomycetes, orange	0	0	0	0	0	0
313	Conant 1004 Lacoz asteroids A. asteroides	0	0	0	0	1A	0
314	Wheeler, Conant 979 A. asteroides	2G	0	0	0	0	0
Total organisms 3		—	—	—	—	—	—
Total animals infected		2	0	0	0	1	0
Total animals given inoculations		12		12		12	

* A indicates acute suppurative; G, granulomatous.

a better understanding of the host-parasite relationship following invasion of the tissue by acid-fast organism; (3) to broaden our conceptions of the reaction potential and its activation in the cells of the reticuloendothelial system, hence our understanding genuine granuloma in its various forms.

Materials and Methods

Three hundred thirty-eight acid-fast organisms were accumulated with the help of Dr. Selma A. Waksman, Dr. William A. Hagan, Dr. Joseph D. Aronson, Dr. Norman A. Conant, and other colleagues. Of these, 208 were selected for intensive study. The study was carried out in two parts.

PART I.—Each organism was studied for cultural characteristics, special emphasis being placed on the following characteristics:

1. Gross appearance on beef extract agar, Dubos' agar, Sabouraud's agar.

2. Cultural reactions and growth in beef extract broth, Sabouraud's broth, and Dubos' broth.

3. Acid-fastness as determined by resistance to decolorization when subjected to different concentrations of acid-alcohol for different periods of time.

4. Morphologic characteristics of the living, growing, unstained organisms as determined by use of the Van Tieghem cell mount, with special emphasis on the occurrence or absence of true branching.

5. Ability to utilize paraffin as the sole source of carbon as indicated in cultures in which a glass rod coated with sterile paraffin was suspended in a flask of carbohydrate-free Czapek's broth.

6. Resistance to digestion by different concentrations of sodium hydroxide.

7. Growth characteristics in litmus milk.

PART II.—Four guinea pigs, four rats, and four mice were given inoculations subcutaneously of 0.2 cc. of a saline suspension of organisms in concentration of 30 organisms per oil-immersion field. All animals were killed at the end of 30 days. A complete autopsy was performed on every animal, followed by histologic study of heart, lungs, liver, spleen, adrenal, kidney, testis, skin, hilar lymph nodes, trachea, pancreas, and, in some instances, brain. Each of the 208 organisms was thus introduced into 12 animals. Approximately 8100 sections were studied, in the distribution of 4000 from guinea pigs, 2500 from rats, and 1600 from mice.

Forty-eight hours before the animals were killed the guinea pigs were given a second inoculation with the same number of organisms as in the first inoculation. The purpose of this was to learn whether or not an alteration in the morphology of the reaction to the organisms might occur in a situation resembling natural reinfection.

Histologic sections were usually stained for acid-fast organisms when the character of the lesions indicated this technique. Cultures were made at autopsy when suitable material could be obtained. Smears stained by Ziehl-Neelsen techniques were made when indicated.

Results

PART I (Microbiologic Study).—The data accumulated in the study of these organisms are voluminous and too varied to permit detailed correlation.* For lack of acceptable applicable criteria, it proved too hazardous to carry classification of these organisms beyond that of the original four major groups.

1. *Morphology of Organisms Grown on Beef Extract or Sabouraud's Agar Media.*

All of the organisms originally identified as Nocardia showed branching and the presence of V and Y forms. Eighteen of the twenty-one strains showed the presence of granules, and thirteen exhibited arthrospores. Three of the strains identified as Mycobacterium showed true branching in this media. Eleven of the fourteen strains of Mycobacterium showed V and Y forms. The mycobacteria not showing V and Y forms were M. tuberculosis, M. marpman, M. ranae, M. lacticola, M. leprae, M. chelonei, and M. sp. unidentified. All of the organisms showing V and Y forms are considered by other observers to be saprophytic except M. avium No. 4, M. leprae (Clegg), and M. paratuberculosis. Many of the soil saprophytes showed the V and Y forms.

2. *Van Teighem Cell Mount Preparations.*

Of the 208 organisms, 104 showed no evidence of branching, 99 showed true branching, and in 5 this feature was doubtful. The Nocardia all showed branching. The mycobacteria failed to show this characteristic, except M. lacticola and M. mist and one unidentified species. Of the 164

* Detailed tabulated data on all organisms are available from us on request.

soil saprophytes, 74 showed this feature.

3. *Degree of Acid-Fastness.*—Many organisms were strongly acid-fast, and many were weakly acid-fast. One may say from our study that the Nocardia are weakly acid-fast and the mycobacteria are strongly acid-fast. However, exceptions may be noted. Good examples are N. mexicanus from the group of Nocardia in this study and the organism of our previous study, N. intracellularis. The organisms of the saprophytic group were varied with respect to acid-fastness.

4. *Cultural Characteristics and Growth.*—In general no specific correlations could be made from these data. The characteristics of growth of the organisms and their reactions in the various media employed could not be grouped according to any useful pattern beyond that which is basic for the original four major groups.

5. *Ability to Utilize Paraffin as a Source of Carbon.*—The results in this part of our study indicate that 19 of the 21 strains of Nocardia possess this characteristic strongly, while most of the strains of Mycobacterium, like the other two strains of Nocardia, exhibit this to a minimal degree if at all. As in other contrasts, the soil saprophytes show a scattering of this type of activity, with some strains behaving like strains of Nocardia and others, like strains of Mycobacterium.

6. *Resistance to Digestion by Sodium Hydroxide.*—The mycobacteria and the nocardiae showed little resistance, and most strains, none at all. The reaction of the soil saprophytes was variable.

7. *Growth Characteristics in Litmus Milk.*—The mycobacteria uniformly showed growth but with indefinite alteration of the medium. Where changes in reaction were noted, the tendency was toward alkalinity. With the nocardiae some strains showed coagulation and the formation of a pellicle. Some produced alkaline, and others, acid changes. In none did the reaction remain unchanged. The soil saprophytes provoked a variety of changes. The majority produced an alkaline reaction and no coagulation or pellicle formation.

PART II (Pathologic Anatomic Study).—The findings at autopsy in the animals given inoculations consist of a variety of lesions. Only the inflammatory reactions considered to be due to the inoculated organisms are pertinent to our problem. In most instances the inflammatory nature of the lesions of interest was confirmed by demonstration of the inoculated organisms in the tissues by smears, by staining the tissues by the Ziehl-Neelsen and other bacteriologic techniques, or by cultures.

1. *Incidence of Infection.*—The incidence of induced specific inflammatory lesions according to the species of animals and major groups of the organisms studied is recorded in Tables 1, 2, 3, 4, and 5. It is noteworthy that 6 of the 21 species of Nocardia produced no lesions in any of the animals of the three species used and only 1 of the strains of Nocardia produced lesions in all three animal species. By contrast, only two species of Mycobacterium failed to produce lesions in at least one species of animals, while seven species produced lesions in all three animal species. Among the soil saprophytes are many which behaved like the nocardiae and many which behaved like the mycobacteria. These differences were not defined with sufficient clarity to be used as a basis of reclassification of the organisms.

TABLE 5.—*Incidence of Infection in All Animals by Groups of Organisms Studied*

	Total Animals	Total Animals Infected	% Total Animals Infected	% Local Infection Only	% Systemic Infection
Nocardia	252	44	17	9	8
Soil saprophytes	1,968	785	40	38	2
Actinomycetes	36	3	8	8	0
Mycobacteria	240	62	26	25	21
Total	2,496	894	36	33	3

However, it may be noted that the incidence of local infections and that of systemic infection in the group of saprophytes closely parallel the corresponding incidences in the group of mycobacteria, indicating that some of the saprophytes may be in fact mycobacteria.

2. Susceptibility of Animals to Infection.—In Tables 1, 2, 3, and 4 the susceptibility of the different species of animals is shown by indicating the number of animals infected after inoculation with the several organisms under study. The mouse was infected in larger numbers and by more species than the other two animals, especially so in the case of the nocardiae and the soil saprophytes.

All animals showing one or more lesions elsewhere than at the site of inoculation are considered to have had systemic infection. In no instance was there systemic spread in the absence of a lesion at the site of inoculation. It may be noted in Table 5 that 894, or roughly a third, of the 2496 animals of the experiment showed infection at the end of 30 days. Of those infected, 120, or nearly one-seventh, showed a degree of extension to produce a systemic spread. The infection incidence in each of the four groups of organisms is shown in Table 5.

3. Morphologic Reaction to the Inoculated Organisms.—For our immediate purposes we may describe acute suppurative inflammation simply as the outpouring at the site of injury of a mass of polymorphonuclear leukocytes which proceed to attack the injuring agent and result in its destruction or its isolation (Fig. 1). In contrast, granulomatous inflammation may be defined as a reaction of the cells of the reticuloendothelial system to the injuring agent (Fig. 2). A combination of these two cellular reactions constitutes mixed inflammation. (Fig. 3). These simple statements of the matter take no account of the sequences of changes in the morphology and function of the reacting cells in the different types of inflammation which are so important to an understanding of the reaction potential of inflammatory cells to harmful agents and the provoking ability of these agents. These matters have been dealt with in detail elsewhere.[2]

In the same animal species, the reactions provoked by a particular organism usually were found to be consistent. Occasionally different animals of the same species reacted differently to the same organism. Indeed, lesions in a given animal produced by one and the same organism not infrequently differed significantly. These inconsistencies

Fig. 1.—A typical acute suppurative reaction in muscle produced in Guinea Pig 169-3 by a clearly acid-fast soil saprophyte, Hagan R-59. Only polymorphonuclear neutrophilic leukocytes have reacted to the organism. Acid-fast organism stained in the lesion.

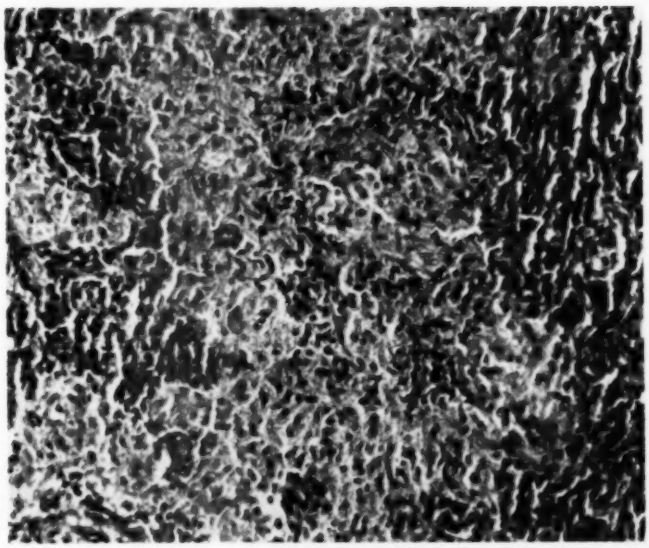

Fig. 2.—A typical epithelioid type of granulomatous reaction produced in the subcutaneous fat of Rat 125-3 by an intensely acid-fast soil saprophyte, Hagan R-122. Acid-fast organisms not stained in the lesion.

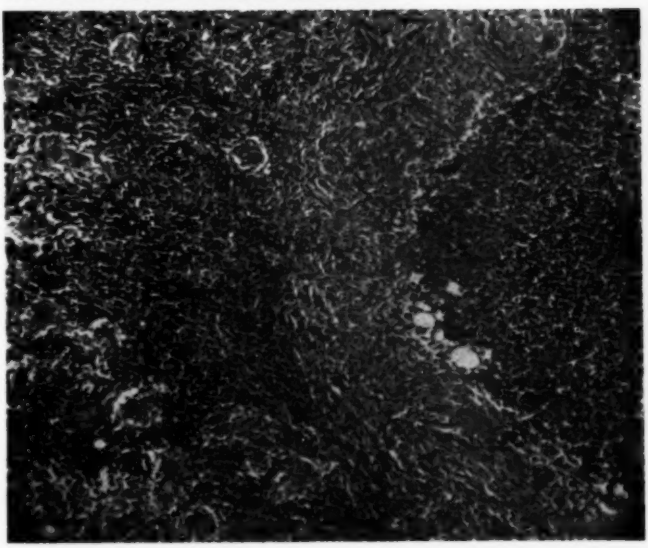

Fig. 3.—One-half of a typical mixed reaction produced in the subcutaneous fat of Rat 127-2 by a highly acid-fast soil saprophyte, Hagan R-68. In the center of the lesion, right, only polymorphonuclear neutrophilic leukocytes appear. The intermediate zone is made up of epithelioid cells. The outer zone, left, consists of clusters of epithelioid tubercules and "lymphoid" cells. Acid-fast organisms stained in the lesion.

were related most conspicuously to the mixed and the granulomatous reactions. Thus the morphology of the lesions produced, although predominantly of the mixed and granulomatous types, could not be said to be specific either for species of animal or for species of organism.

In almost all the animals used in this experiment there was coincidental disease unrelated to that produced by the inoculated organism.

Comment

As previously indicated one of the main objectives of this study was to discover if possible organisms identical with or resembling in their biologic and pathologic properties N. intracellularis originally iso-

lated from an extraordinary case of pure granulomatous disease the specific etiology of which it was proven to be. This we have not succeeded in doing. N. intracellularis thus continues to remain in its unique position among the acid-fast organisms. However, this uniqueness may eventually turn out to rest not in the biologic character of the organism but in a highly specific host-parasite relationship. We have no idea from this study as to what the reactions of the human host would be to most of the organisms which have been the subject of this investigation. Infection of man by those acid-fast organisms among the 208 which are familiar to us, for example, M. tuberculosis and N. asteroides, certainly bears only a superficial resemblance to that produced by N. intracellularis. At the same time there are striking similarities between N. intracellularis and these organisms from the point of view of both biologic properties and pathogenicity for the laboratory animal. From what we learned about N. intracellularis in our previous study of this organism and from what we know now about the 208 acid-fast organisms of many sources under study in this investigation it is quite evident that had N. intracellularis been included in the organisms here being comparatively studied, it would not have been recognized for what it is. All of this, of course, merely emphasizes again the basic fact that the reaction potential of host cells dominates and determines not only whether or not clinical disease will occur but also the specific character of the disease when it does develop after parasitic invasion.

The data in the Tables show at a glance that the 208 acid-fast organisms studied produced lesions in common laboratory animals with few exceptions. These lesions progressed and in many instances were followed by dissemination of the organisms and the development of lesions like those at the inoculation site. In great majority the lesions were of the type we have described as granulomatous and mixed. This predominance was manifest regardless of the type of organism inoculated and at the same time independent of the species of animal given the inoculation. Such a small number of the organisms studied produced frankly acute suppurative lesions that it is permissible to say that the typical lesion produced is not that provoked by ordinary pyogenic bacteria. This, of course, suggests some pathogenic factor or factors common to the several groups. However, when the data derived from our studies are searched for such a common factor, none is found. Not even the acid-fastness suffices. Organisms strongly acid-fast and others with such weak acid-fast properties as to cause us to record them as non-acid-fast or equivocally so at the time of our study (they were all originally acid-fast) differed little in their ability to produce the typical granulomatous or mixed reaction. As was very clear from the histologic studies, the number of organisms present in the lesion was of little importance in determining the character of the cellular reaction. It appears fair to assume that none of the animals given inoculations had had any previous contact with the organisms inoculated, except those few which received a second inoculation. Since the lesions in all, including those receiving a second inoculation, were alike, any likelihood that an altered reactivity of the tissues—allergy, hypersensitivity—might have determined the cytological character of the lesion would appear insignificant. Examination of the cultural data pertaining to the organisms individually or by groups is equally unproductive of a valid common pathogenic factor. Thus we are left with little that can be said about the provocative potential of these organisms than that it must reside in some as yet undisclosed chemical factor which presumably is common to them all though perhaps more abundant or active in one species than in another. Such a situation is, of course, not at all inconsistent with what is common knowledge with respect to certain well-known bacteria highly pathogenic for man.

Concluding Summary

A study of 208 acid-fast organisms from various sources originally classified as Nocardia, Mycobacterium, Actinomyces, or soil saprophytes has been made with reference to their microbiologic properties and their pathogenic potentials for mice, rats, and guinea pigs. The basic objectives of this study were (1) to discover other strains of Nocardia intracellularis, an organism known to produce in man a unique form of fatal granulomatous disease, only a single instance of which has been observed; (2) to gain a better understanding of the host-parasite relationship when acid-fast organisms are introduced into tissues, and (3) to acquire a better comprehension of granulomatous inflammation as a basic pathologic mechanism. The results of this study seem to warrant the following concluding statements:

N. intracellularis remains unique in that no organisms identical with it have been recognized in this large group of acid-fast organisms among which it might have been expected to be found. Its natural habitat, furthermore, remains to be demonstrated.

Many acid-fast organisms of different classification and from different sources generally thought to be nonpathogenic actually produce not only local lesions but progressive disease in common laboratory animals. Under conditions yet to be determined they, like N. intracellularis, may be expected to produce progressive, even fatal, disease in man.

Surprisingly, the pathogenicity of the organisms designated soil saprophytes for mice, rats, and guinea pigs significantly surpasses that of the other groups. This suggests that the soil may be a highly important reservoir of organisms potentially dangerous for man.

All types of inflammatory reaction—acute suppurative, genuine granulomatous, and mixed—are provoked by acid-fast organisms of the varieties included in this study, but the granulomatous and mixed reactions are the commonest regardless of the species of organism or host. This suggests the existence of a pathogenic factor which is common to all species of organisms, but this study does not indicate what this factor may be.

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The Effects of Hormones upon Hepatic Glycogenesis

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The adrenal cortical glycosteroids facilitate or in some manner participate in the process of hepatic glycogenesis. The 11-oxycorticosteroids have even been referred to as the "glycogenic hormone."[1] This property of the steroids assumed importance during our examination of glycogenolytic agents upon carbohydrate "turnover" rates in tissue slices from animals prepared to saturation with these hormones. A knowledge of the participation of insulin, if any, in this adrenocortical function was of similar importance. Information available from in vivo experiments was found inadequate.

As large a variety of controlled circumstances as possible was devised by which the relationship of insulin to this steroid function could be examined and under which effects responses might express themselves as a trend or pattern. The greater the number of animals subjected to each experimental circumstance, it was believed, the less the likelihood of failure to recognize any such trends. However, these large numbers of animals were not used in some of these procedures.

Experimental Procedures

Sprague-Dawley strain male rats were used, within a weight range of 250 to 350 gm. A stock colony of diabetic animals was established by the subcutaneous injection of alloxan, 17.5 mg/100 gm. of body weight, in a 2.8% aqueous solution. The permanency and satisfactory severity of their diabetes were verified. Those with a high daily glycosuria and polyuria of several weeks' duration were selected and were starved for 30 hours. Animals in which blood glucose concentrations had fallen close to normal levels were discarded. From 200 rats with adjudged satisfactory diabetes, 28 were removed which failed this criterion and were regarded as imperfect examples.

A large group of normal and diabetic rats was fed Purina Chow ad libitum throughout the period of observation or experiment on them. All animals ate plentifully, and any appearing listless or whose metabolism cages showed only a small amount of collected food debris over many hours of time were eliminated from further use. A duplicate normal and diabetic group was maintained by force-feeding the high-carbohydrate diet compounded and used by Ingle.[2] Cortisone acetate (Merck) was given to approximately half the number of animals, normal and diabetic, in both groups. The daily amount of 5 mg. by intramuscular injection was increased gradually to a daily maximum of 15 mg.[3] Other diabetic rats, chosen from the free-feeding group, received daily injections of protamine zinc insulin, either alone or concomitantly with injections of cortisone acetate; the procedures and principles of care in this circumstance were those described by Sturtevant.[4] A third series of normal and diabetic animals was maintained under starving conditions for 48 hours. Hydrocortisone (Merck) in 2 mg. amounts at four-hour intervals was given by intramuscular injection to all but the control animals throughout this period. Finally, a smaller colony of partially depancreatized rats was force-fed the high carbohydrate diet during both a control and a lengthy cortisone administration period.

Multiple liver samples were obtained from all animals after decapitation; several rapidly made and distributed snippets of the organ were dropped into liquid nitrogen.

Healthy male mongrel dogs were allowed an unlimited amount of Nutrena dog food and one pound of fresh mixed meat each day. Alloxan diabetes was induced in some of them by the intravenous injection of 70 mg. of alloxan per kilogram as a 5% solution. The satisfactory and permanent nature of the diabetes was ascertained by daily blood and urine glucose estimations for one week after a postinjection "adjustment" interval of two weeks. One series of normal and diabetic dogs was then subjected to an "extended biopsy" procedure; another series, to the "acute

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biopsy" procedure. The former consisted in the frequent removal of liver biopsy specimens over an extended period of time from some and the daily preparation of others, with injections of cortisone interspersed with repeated liver biopsies. The commencing dose of cortisone was 25 mg., increasing quickly to a daily maximum of 75 mg. for a 10 kg. dog. The "acute" procedure comprised the removal of multiple control liver biopsy specimens from normal and diabetic dogs previously starved for 48 hours and from similar dogs which during the last 12-hour period of starvation had been repeatedly given large intramuscular amounts of hydrocortisone. While the light anesthesia required for the biopsy persisted, 50 gm. of glucose was given slowly by duodenal intubation. Blood glucose values were determined at short regular intervals upon samples from the femoral vein, and absorption of glucose was verified. At the end of two hours, the second, and final, series of biopsies was done as rapidly as possible through small abdominal incisions, and the excised tissue was placed in liquid nitrogen. During these procedures attention to all the features important to the operative welfare and response reliability of the dogs followed the text of the complete discussion by Bodo.[5,6] In very few instances, an intravenous drip administration of prednisolone * throughout the period of glucose feeding replaced the hydrocortisone preparation. The number of trials of this intravenous procedure is presently inadequate but allows a reserved demonstration of the pattern trend obtained.

The tissue glycogen concentrations were determined by the anthrone reagent method; the trichloracetic acid (TCA)-soluble fraction and the total (alkali-digestion) were estimated.[7,8] The quantitative procedures of Nelson and Benedict were used for the determinations of blood and urine glucose, respectively; Rothera's test determined the presence of urinary ketones. Histologic localization and assessment of tissue glycogen utilized the periodic acid-Schiff technique following the Altmann-Gersh freeze-drying process.

Results Obtained

The pattern distinctions obtained are made more obvious by their graphic expression in Charts 1 to 6. It was readily apparent that the liver of the fed diabetic

* Supplied for this purpose as prednisolone 21-phosphate, PC 1382-A52, by Dr. Elmer Alpert, Clinical Research Laboratories, Merck Sharpe & Dohme, Division of Merck & Co., Inc., West Point, Pa.

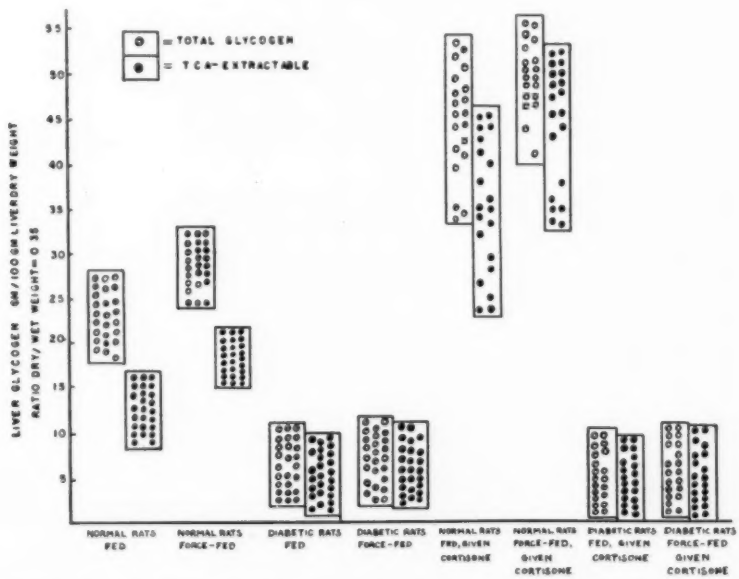
= TOTAL GLYCOGEN
= TCA-EXTRACTABLE
LIVER GLYCOGEN GM/100 GM LIVER DRY WEIGHT
RATIO DRY/WET WEIGHT = 0.35
NORMAL RATS FED
NORMAL RATS FORCE-FED
DIABETIC RATS FED
DIABETIC RATS FORCE-FED
NORMAL RATS FED, GIVEN CORTISONE
NORMAL RATS FORCE-FED, GIVEN CORTISONE
DIABETIC RATS FED, GIVEN CORTISONE
DIABETIC RATS FORCE-FED GIVEN CORTISONE

Chart 1.—Hepatic glycogen values of normal and alloxan-diabetic, fed, and force-fed rats, with and without cortisone administration. Open and solid circles indicate the mean glycogen value of multiple liver samples from each animal.

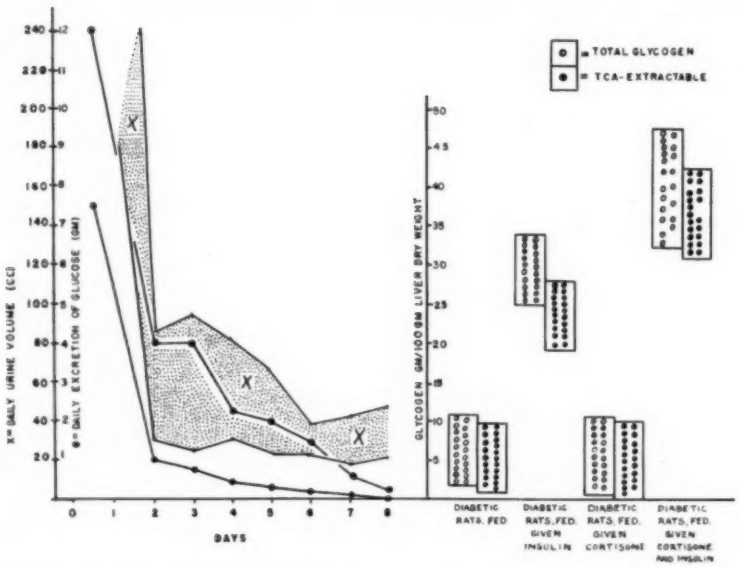
= TOTAL GLYCOGEN
= TCA-EXTRACTABLE
X = DAILY URINE VOLUME (CC)
= DAILY EXCRETION OF GLUCOSE (GM)
GLYCOGEN GM/100 GM LIVER DRY WEIGHT
DAYS
DIABETIC RATS, FED
DIABETIC RATS, FED. GIVEN INSULIN
DIABETIC RATS, FED. GIVEN CORTISONE
DIABETIC RATS, FED. GIVEN CORTISONE AND INSULIN

Chart 2.—Alloxan-diabetic rats receiving insulin and cortisone. The bar graph shows the glycogen concentration differences between the steroid-treated diabetic groups with and without insulin therapy. Open and solid circles indicate average glycogen values of multiple samples from each rat. The conventional graph is a composite of the daily ranges of urinary volumes and glucose concentrations, respectively, of the steroid-treated diabetic rats. It shows their diminishing amounts during the period of insulin therapy.

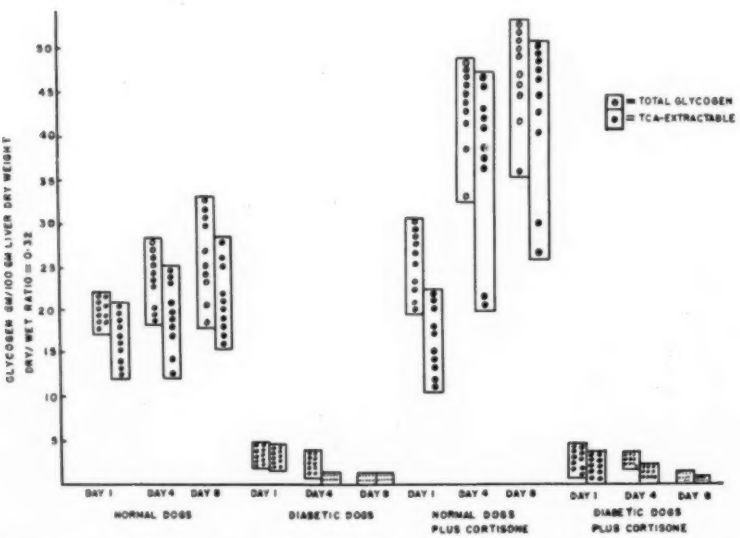

Chart 3.—Normal and alloxan-diabetic dogs given injections of cortisone. Patterns of hepatic glycogen values obtained with the "extended biopsy" procedures. Open and solid circles indicate the mean hepatic glycogen of several samples from each dog.

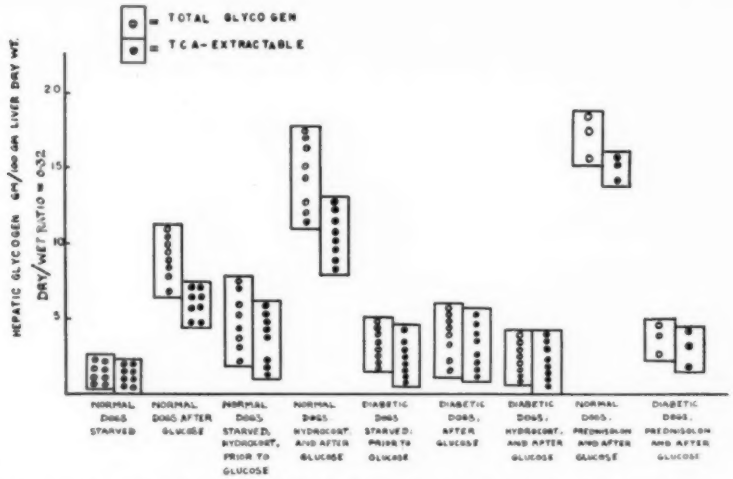
= TOTAL GLYCOGEN
= TCA-EXTRACTABLE
HEPATIC GLYCOGEN GM/100 GM LIVER DRY WT.
DRY/WET RATIO = 0·32
20
15
10
5
NORMAL DOGS STARVED
NORMAL DOGS AFTER GLUCOSE
NORMAL DOGS, STARVED, HYDROCORT. PRIOR TO GLUCOSE
NORMAL DOGS, HYDROCORT. AND AFTER GLUCOSE
DIABETIC DOGS, STARVED: PRIOR TO GLUCOSE
DIABETIC DOGS, AFTER GLUCOSE
DIABETIC DOGS, HYDROCORT. AND AFTER GLUCOSE
NORMAL DOGS, PREDNISOLON AND AFTER GLUCOSE
DIABETIC DOGS, PREDNISOLON AND AFTER GLUCOSE

Chart 4.—Normal and alloxan-diabetic dogs: Results of the "acute biopsy" procedure. Patterns of hepatic glycogen values after intraduodenal glucose, with and without the administration of glucocorticosteroids. Open and solid circles indicate the mean glycogen value of several liver samples from each animal.

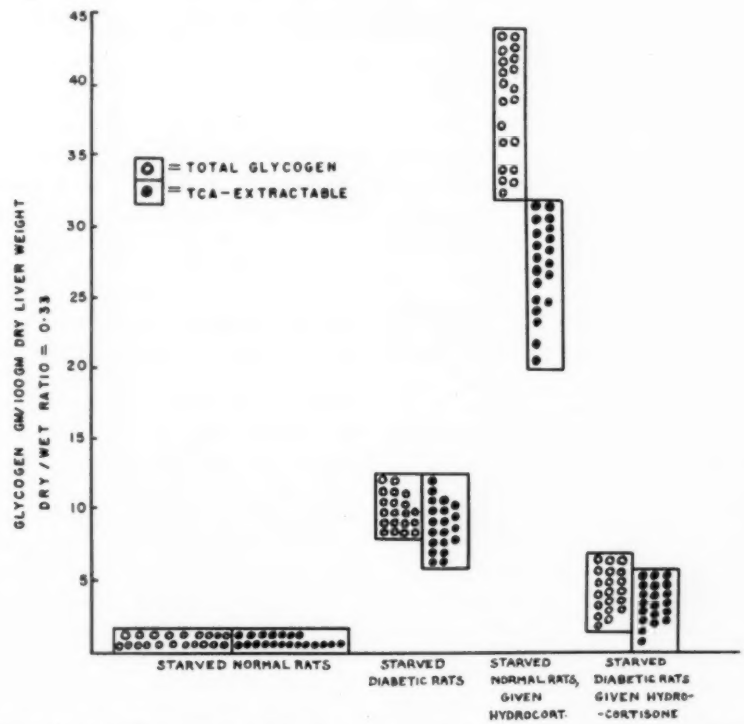

Chart 5.—Starved normal and alloxan-diabetic rats: Patterns of hepatic glycogen values with and without glucosteroid preparation. Open and solid circles indicate the average value of glycogen in many liver samples from each rat.

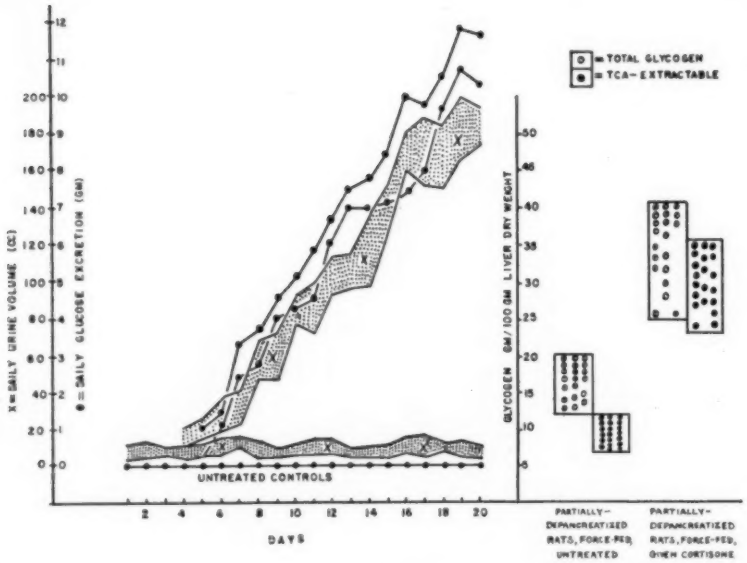

Chart 6.—Partial-pancreatectomized rats, force-fed and receiving cortisone. The bar graph shows the pattern of their hepatic glycogen concentration. Open and solid circles indicate the average glycogen concentration of multiple liver samples from each rat. The conventional graph is a composite of the daily ranges of urinary volumes and glucose excretion, respectively, of the rats during steroid preparation.

animal does not possess the concentration of glycogen discovered in the normal animal. This was found true in the force-fed colonies as well as the free-feeding groups. Comparisons of these quantity patterns can be seen from the bars of Charts 1, 2, and 3.

It was equally apparent that the glucosteroids potentiate the deposition of hepatic glycogen in normal animals. This was particularly well exemplified by the experimental procedures upon the rat and the "extended biopsy" method upon the dogs (Charts 1 and 3). However, the "acute" procedures upon the latter animals were somewhat less emphatic in demonstrating this (Chart 4). There was approximately a twofold increase in the concentration of total liver glycogen of the well-fed rat receiving cortisone above that of the fed normal animal. The contrast of this pattern with that of the diabetic was undeniably plain. The liver glycogen of the diabetic was much below that of the normal and fed rat, sometimes almost close to the vanishing point. The administration of cortisone, no matter how long continued and in what large doses, made little impression upon the hepatic glycogen concentrations of the diabetic animal. Indeed, there was observed a slight, but possibly insignificant, tendency for these already low diabetic levels to fall still farther when cortisone had been administered. The introduction of insulin therapy for the colony of diabetic and cortisone-treated rats effected the pattern changes depicted on Chart 2. Liver glycogen quantities were emphatically raised; they approached the elevated concentrations typifying the normal animal receiving the steroid injections. The descent of daily urine volumes and glucose concentrations during this period of use of insulin is graphically included on this same Chart.

The results obtained with intravenous prednisolone in the "acute" biopsy technique, although numerically inadequate, are

depicted in Chart 4. The response patterns with the few animals used were unmistakably like those obtained with the comparable longer procedure. Any increment that may have occurred of the sparse glycogen of diabetic dogs receiving glucose, and wrought by the steroid infusion, remained undetected. In distinction, greater amounts of glycogen were formed by the liver of the normal animal under the influence of the steroid than by the normal animal receiving glucose alone.

Starvation of the normal rat reduced its hepatic glycogen profoundly. Chart 5 shows its different quantity pattern, the component values of which agree with the reduced concentrations previously reported. Starvation of the diabetic rat, however, brought about no further significant descent of the proportionately low feeding values. These latter now assumed the greater of the two comparative concentrations. However, injection of the glucosteroid increased remarkably the hepatic glycogen of the fasting normal animal (Chart 5). The fasting glycogen values of the diabetic remained uninfluenced by this additional hormone circumstance.

A number of diabetic rats manifest ketonuria, sometimes to a profound degree. The phenomenon was accentuated or made more persistent by the administration of cortisone; its relevance is discussed below. It suffices that none of these animals manifesting ketonuria during the control procedures or during the actual experimental tests showed clinical distinctions from the nonketonuric rats. Their food intake and wastage were not noticeably different. No animal was lost or discarded because of clinically apparent ketosis or coma once the time interval required for the diabetic rat's metabolic adjustments and acceptability had passed. During this preparatory interval, however, sickness and coma reduced the number in the diabetic colonies appreciably.

The histologic use of the periodic acid-Schiff technique was of limited value and possibly questionable significance in comparison with chemical quantitation after glycogen extraction. Its use has, in general, previously confirmed the accentuated deposition of glycogen in the cortisone-treated normal rat.[9] However, in one reported instance employment of histochemical procedures described as "quantitative" has denied the increase of hepatic glycogen under steroid stimulation.[10] It was effective in this present instance only to show the very diminished glycogen in the liver cell of the starved normal and some few of the alloxan-diabetic animals. No helpful purpose was served in all of the glycogen-rich examples except merely that of confirming plentiful glycogen. It did not distinguish, for example, between the hepatic glycogen concentration of a normal well-fed animal and of a similar animal receiving cortisone. No abnormal cell situation of the hepatic glycogen was seen in any of the diabetic preparations. We were led to conclude that microscopical tissue techniques may prove misleading and perhaps responsible for erroneous conclusions when used as the sole indicator.

Comment and Conclusions

The liver has long occupied a central and commanding position in the mosaic of carbohydrate metabolism studies. It is the situation of the principal labile store of carbohydrate in the body, ordinarily containing the highest concentration of glycogen. The latter is present in the cells, however, in amounts which are small compared to the total energy needs of the organism. Thus, it is expected that the stored glycogen is in dynamic equilibrium with these needs, undergoing rather rapid utilization and replacement. Most conclusions about the processes concerned in the regulation of this equilibrium must be inferred from the changes in glycogen quantities present in the tissues under different and clearly definable circumstances. Several normal chemical mediators of carbohydrate metabolism may sway the behavior of this carbohydrate reserve in one

direction or the other. It has been wisely remarked that any undertaking is somewhat hazardous which tries to assess the roles of hormones in carbohydrate metabolism from observations of their effects on tissue glycogen concentrations. However, there are good reasons why such attempts are worthwhile. The amount of glycogen present at any time is the resultant of many processes; the availability of carbohydrate from the diet or its formation from other substances, the unhindered rate-controlled absorption of glucose into the cell, its phosphorylation, and its oxidation to other compounds are but some of these.

Information about the glycogen "turnover" rates of normal tissues is scant and unsatisfactory; the tissues of the hormonal-imbalanced organism, moreover, extend additional problems. Nevertheless, there are definite, though general, quantity-circumstance patterns for hepatic glycogen, despite what may appear in detail as a confusing multiplicity of variables. Such patterns, clearly dependent upon the most stringent observance of the selected circumstance, constitute reliable evidence for one's concepts and their argument. The complex of the dynamic norm between the interchanges of free and reserve carbohydrate invokes the participation of several hormones in small or large degree. Epinephrine and glucagon, promoting a speedy hepatic release of labile carbohydrate, exemplify its glycogenlytic fraction. A puzzling reaction of glycogen-rich tissues to these agents prompted the present study of glycogenesis. Rabbits and rats had been prepared with daily injections of hydrocortisone or cortisone, ensuring livers extremely rich in glycogen for supplies of liver slices. These latter surprisingly responded poorly indeed to glucagon, the percentage glucose increase in the incubation media being below the yield of other liver slices from an unprepared source. Epinephrine responses likewise were only fair.

The thought occurred that perhaps this circumstance and its antecedents might be related to another in which hepatic glycogen is present in abundance in some unquestionable instances of severe clinical diabetes. This exceptional and seemingly paradoxical association is without a satisfactory explanation. Recently studied examples have been described in which continuous glucagon administration failed to influence the blood glucose of diabetic patients, whereas repeated liver biopsies confirmed abundant tissue glycogen.[11] These clinical examples are not to be confused with von Gierke's disease; they do not fulfill the criteria defining this clinical condition.[12] The thought was extended that perhaps involved in this problem were some distinctions between glycogen fractions in their physical properties and physiologic behavior. Large and consistent differences have been found between the amounts of glycogen extractable from fresh muscle tissues by trichloracetic acid and those resulting after alkali digestion. In a variety of experimental circumstances the TCA-extractable glycogen has been the mobile fraction, the nonextractable, or "bound," glycogen remaining relatively constant. Although much of this work has been upon muscle tissue, an assumption seemed reasonable that the glycogen of liver may exist and respond in like fractions. However, further evidence was required, particularly from sources where hormonal influences were of foremost concern. Thus, reasons evolved to contemplate the original glycogenic process and to observe at least the more obvious features of glycogen accumulation. Two principal hormone reactants within these processes, namely, the glucocortico-steroids and insulin, were of greatest interest.

The liver is predominantly implicated as the organ whose role in carbohydrate metabolism is influenced remarkably by the adrenal cortical hormones.[13] The facilitation or stimulation of hepatic glycogenesis by these steroids is well acknowledged [14-17] and is also the basis of a biological assay for their potency.[18-21] Long and his col-

leagues,[16] for example, found that injections of adrenal cortical extract into mice to which a high carbohydrate diet was fed resulted in a large deposition of liver glycogen, sometimes to a concentration level as high as 12 gm.%. The continued administration of cortical extracts or pure hormones frequently results in a temporary, though sometimes severe, diabetes. This characteristic, exemplified by hyperglycemia and glycosuria, still retains the relationship of liver glycogen values exceeding those of the fed normal animal.[2,15,16,22] The high-carbohydrate diet is actually unnecessary to reveal the glycogenic propensities of the steroids, as their ability to increase considerably the hepatic glycogen of fasting animals has been demonstrated.[13] The starved animal can accumulate 10 or 20 times as much glycogen in a 12-hour period; although the logical presumption would be a greater need for carbohydrate, the organism appears unable to utilize this accumulation in a normal manner.

An opinion exists that the cortical hormones are not endowed with a direct action on glycogen production or storage. These latter are incidental to and the result of an accelerated gluconeogenesis by which means additional amounts of glucose are supplied for use in energy production.[23] Blood glucose concentrations may be elevated in consequence and surplus glucose stored as glycogen or fat. Inhibition by the glucosteroids of certain phases of carbohydrate utilization has also been proposed[13,24] which, though seeming somewhat paradoxical to the purpose of gluconeogenesis, may further the amount of glucose available for conservation as glycogen.

A direct effect upon the accumulation of hepatic glycogen has long been attributed to insulin. It is remarkable that with plentiful evidence of carbohydrate storage by dogs given insulin therapy, Best should have considered originally an alternative explanation for the dramatic lowering of blood sugar. "We knew that the liver failed to store glycogen and the respiratory quotient failed to rise when sugar was given to a depancreatized dog. Actually our first thought on the action of insulin was that it caused more sugar to be burned."[25] It is the general concensus of opinion that this association between hepatic glycogen and insulin adequacy is a very real one, despite some data which would deny or minimize it.[5,26] Russell[27] believes that unequivocal experimental support is still lacking for proof that insulin deficiency need be equated with liver glycogen depletion but agrees that insulin given with glucose tends to increase the rate of hepatic deposition. The recent innovation of hepatic vein catheterization in man has been the means of showing that insulin not only reduces the output of glucose of the liver but also promotes its hepatic withdrawal from the circulating blood.[28]

Animals deprived of their pancreases generally have a low hepatic glycogen concentration, which can be restored toward normal upon administration of the hormone.[5,6,29,30] Bodo and his colleagues[6] reported a maximum gain of only 1 gm.% by the glycogen-free liver of depancreatized dogs after glucose feeding. Best's[25] example of a glycogen value of over 20 gm.% under similar circumstances, but with the addition of insulin, is an emphatic contrast. Since the use of alloxan to produce diabetes mellitus there have been conflicting reports of low, normal, or high hepatic glycogen in diabetic animals. In general, moderately low values appear to pertain in well-fed animals when compared with their normal and equally well-fed counterparts. Approximately one-third of the normal, or control, glycogen values obtained in the experimental series of Winternitz and Lattanzi.[31] It would seem that the magnitude of glycogen deposition appears roughly to vary inversely with the severity of the alloxan-diabetic state—more explicitly, perhaps, with the adequacy of the diabetic preparation.[26,32] The belief is also fairly general that liver glycogen is diminished in human diabetes mellitus; a profound depletion,

however, need not necessarily apply.[33,34] However, exceptional and thought-provoking instances of remarkably high glycogen storage have been previously referred to.[11,35,36] Some failures to discover glycogen quantity differences between the human diabetic and the normal[37,38] may be merely a reflection of the similar parallelism between the "degree" of diabetes and glycogen concentrations of the experimental animal.

This lack of glycogenic response of the diabetic animal is pointedly reminiscent of its behavior when supplied with glucose and would therefore support Verzar's[23] concept that the principal effect of cortisone administration is the provision of glucose. Indeed, it would seem fair to conclude that the term "glycogenic" as applied to the adrenal glucosteroid hormones cannot be used in any restricted sense of meaning and is inaccurate unless there be considered a concomitant adequacy of the secreted hormone insulin. The provision of the latter for these purposes need only be small or, more correctly, supplied by a small amount of pancreatic islet tissue, a feature demonstrated by the hepatic glycogen values of the partially depancreatized rat (Chart 6). Although approximately 90% of the pancreas had been removed from every rat in this experimental group, the hepatic glycogen concentrations of the control and the cortisone-treated animals resembled entirely the patterns from rats with normal pancreases.

The purpose in using this operative procedure evolved from a consideration that the "insulin adaptability" to continued doses of cortisone may possibly be broken down entirely, that it might be feasible to create during this administration the circumstance of a completely insulin-inadequate preparation. The criterion would be a significant change in the pattern of the liver glycogen concentrations from that of the normal cortisone-treated animal to one like that of an alloxan-diabetic under similar hormone stimulus. Thus, at some period during the administration of the cortisone there would result a descent of the glycogen values from the high plateau of the normal to the extremely low values of the insulin-deficient rat. This phenomenon was never observed. It would perhaps have been possible to remove more portions of pancreatic tissue, almost to complete pancreatectomy if this were feasible, but further efforts were not continued at this time. Nevertheless, an equally impressive purpose became exemplified; the function of a much reduced islet-cell volume only is required to effect the hepatic glycogenic potentialities of cortisone. Ingle[15] and his colleagues observed a small decline in the high plateau of hepatic glycogen values in normal cortisone-treated rats, occurring toward the end of a 28-day observation period. They believed it represented an "adaptation" to hypercorticalism; a depleted glycogen storage space or poor glycogenic function of the hepatic cells with the onset of their fatty degeneration was also suggested. A progressive and relative hypoinsulinism could still be reasonably entertained but would be difficult to support by virtue of the factors and technical necessities recounted above. It must be appreciated that the selected criterion was necessarily a change to much lower concentrations of hepatic glycogen; familiar criteria for defining the diabetic state would not have applied to this particular circumstance. Hyperglycemia and intense glycosuria are features occurring in the normal animal during prolonged administration of potent glucosteroids. The dose of only 5 mg. of cortisone daily resulted in a daily glucose excretion of 5 to 7 gm. by the normal rat[2,15,39]; Lazarow and Berman[22] obtained values ranging up to 5 gm. per day during a 15-day period with an injected 10 mg. amount of cortisone daily. Similar figures obtained in the present investigations. The interest and applicability of this problem are intensified when the established conclusion is considered that the cortisone-diabetic state of the partially depancreatized

rat is more intense or dramatic than the same state in the normal rat.[16,40,41] Some of our partial-pancreatectomy rats receiving cortisone excreted as much as 10 gm. of glucose daily and possessed blood sugar values elevated above 300 mg.%. By these criteria they were equal to the most satisfactory alloxan-diabetic animal; yet, despite this accordance with the severest diabetic state, hepatic glycogen values were tremendously raised.

Glycogenesis is possible to some extent in the complete absence of insulin and is demonstrated by the rather perplexing response of the alloxan-diabetic rat to starvation. The liver of the normal animal starved for 24 to 48 hours is almost depleted of glycogen; this is retained by the diabetic animal in fairly substantial amounts. There are no previous decisions as to whether or not the retained glycogen in the diabetic differs in any particular, either physical or chemical, from that of the normal organism or if an abnormal intracellular situation of the carbohydrate extends an added distinction and possible explanation. This is a circumstance in which, it seems, the adrenal steroids participate; removal of the adrenals reduces the moderate glycogen stores to the low levels of the starving normal rat. Its cognizance, physiologically, is seriously influenced by cautions such as Lukens'[42]: "Certain processes . . . can be reduced to diabetic levels by fasting, and . . . conditions under which carbohydrate is normally well utilized must be employed to demonstrate the significance of insulin deficiency." An explanation of its mechanism that cites merely the "glycogenic" activity of the adrenocortical secretion seems inadequate. Also inadequate or equivocal is the evidence incriminating seriously diminished glucose utilization by somatic tissues in the starved diabetic animal and thereby, perhaps, permitting its undisturbed retention as glycogen. There are reasons to imagine that the extremely important function of fat synthesis in the conservation of carbohydrate may actually take precedence over glycogen storage functions of the liver cell and that the comparative absence of glycogen from the liver of the starved normal animal may be its demonstration. In complete insulin inadequacy fat synthesis is seriously hindered; the "unopposed" steroid activity in the diabetic animal would further augment this depression.[43,44] Theoretically, carbohydrate conservation by glycogenesis may then become apparent. However, opposed to such thinking are the findings that fat synthesis can be reduced to diabetic levels in a normal animal merely by prolonged fasting.[42]

Two possibilities must be mentioned which could be considered to reflect upon liver glycogen concentrations under most of the experimental circumstances utilized. One is the interpretation that alloxan, by chemotoxic means, may seriously impair the glycogenic function of the hepatic cell or its chemical responsiveness to the adrenal glucosteroids. The other is that an excessive production of ketones, as evidenced by their urinary excretion, may seriously and adversely influence liver glycogen quantities. The former requires barely a brief consideration and is dispelled by recognition of the alloxan-diabetic rat's ability to accumulate glycogen within its liver cell and, to a large degree, merely by the administration of exogenous insulin. Steroid receptivity is likewise manifest under the same circumstance. The small colony of diabetic rats receiving injections of insulin supplied a significant link in the demonstration of the necessity of insulin for the glycogenic activity of adrenal glucosteroids. This group showed that no significant differences exist between its patterns of liver glycogen and those of a well-fed normal group and with or without the added hormone circumstance of a potent glucosteroid. The concept of a causal relationship between hepatic glycogen depletion and ketone-body overproduction has been widely held.[33-35] It appears, however, that the development of ketosis in diabetes is determined by other factors as well as, or

instead of, liver glycogen stores. Whether fasted or fed, diabetic rats may have elevated blood ketones coexistent with substantial liver glycogen reserves.[32,46] Tuerkischer [47] noted that liver glycogen values did not fall until his diabetic rats had reached the terminal stages of coma, and he concluded that this fall was symptomatic of coma, rather than of diabetes per se. Bondy and his colleagues [35] found high liver glycogen concentrations in some human diabetics with severe ketosis. Vallance-Owen [36] also described normal to high glycogen values in the livers of patients who had died in diabetic coma without insulin therapy. This author likewise concluded that the customary glycogen depletion was secondary to other aspects of diabetic coma, such as inanition or vomiting. In these present studies the patterns of hepatic glycogen behavior in those diabetic but well rats exhibiting 3+ or 4+ ketonuria for many days differed in no respect from those of diabetic rats without this phenomenon.

Finally, the charted results show that in the diabetic preparations the TCA-soluble glycogen more approaches the levels of the total, or alkali-digest, glycogen. This applies not only to the alloxan-diabetic but to the steroid form of diabetes in the normal animal. A relative depletion of the residual, or "bound," fraction, whatever this latter may actually mean in biological terms, becomes apparent. If this trend is true and has significance, then certain problem interpretations, initially considered with possible relationship to the glycogen fractions, have not been supported or even simplified. The TCA-fraction customarily regarded as the "labile" one comprises the relatively greater proportion of the total glycogen in the diabetic animal and exists in considerable proportionate concentration in the liver of the normal but steroid-diabetic animal. Clearly, a failure of glycogenolytic-factor activity upon this large store of glycogen cannot be considered further as responsible for a relative lack of a labile variety of carbohydrate.

In summary, one can barely avoid extending the results and interpretations expressed to human diabetes, with no more than the interested contemplation this would imply. Clearly, with absolute insulin inadequacy, liver glycogen values, with one exception, are below the normal for the particular circumstance; they cannot be elevated by either food or glycogenic stimuli to a degree remotely resembling that of the insulin-adequate organism. There is no confusion between these extremes of circumstance-response. Starvation introduces the exceptional feature, when it may appear that the diabetic has glycogen values equal to and exceeding some of the values of a dietary inadequate but normal organism. Simplified, this must be little more than an additional expression of the diabetic's ability to retain a "basal" value of glycogen throughout fed to starving extremes; it is the normal organism that can be seen as the "sensitive" variable and whose glycogen values are so very responsive to changes of circumstance. One is thus forced to wonder with what awareness these latter have been regarded in the compilation of normal estimates of human hepatic glycogen. Although there is demonstrable resistance to exogenous insulin in steroid diabetes, it is yet unknown whether or not the islets themselves are secreting subnormal, normal, or supernormal quantities. Nevertheless, diabetes of this origin can presumably be regarded as a relative insulin-inadequate diabetes, the inadequacy, as either quantitative or functional. The latter is used in the sense of a poorly understood prevailing chemical inhibition or antagonism, and the former, an exhausted secretory capacity of normal islet tissues under intense steroid overactivity or their actual numerical reduction. Clinical evidence suggests that where the "functional reserve" of the insulin-producing mechanism is depleted, a more intense form of diabetes is elicited in the presence of an excess of

adrenal glucosteroids.[48] It would be interesting to know the hepatic glycogen concentration of diabetics clinically within this category; one can but surmise that they would be raised, and perhaps even to great levels.

Summary

A variety of experimental circumstances has been used to examine the hepatic function of glycogenesis. One of its objects was to determine or clarify relationships between the actions of insulin and adrenal glucosteroids within this physiologic process. Augmentation of glycogenesis by the administered corticosteroids was obtained only in the presence of insulin, of either endogenous or exogenous origin. The absolute insulin-inadequate animal was incapable of this response. Only with this qualification applied can the glucosteroids be precisely considered as hepatoglycogenic. A prominent degree of glycogenesis was possible under circumstances in which insulin supply or its functional efficiency was reduced. Interpretation implies the satisfying of glycogenic needs in the midst of manifestations of "somatic" insulin inadequacy. Toward this accomplishment the insulin secretory tissue could seem anatomically well placed.

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Renal Agenesis and Meningocerebral Angiomatosis

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We have recently studied an infant with bilateral renal agenesis and unusual meningocerebral vascular alterations. The purpose of this report is to explore the relationship of renal agenesis, the facies said to be characteristic of renal agenesis, and developmental vascular alterations of the cerebrum and face.

With the exception of a case of Köhler,[1] in 1940, there have been no previous reports of renal agenesis occurring with angiomatous malformations of the cerebrum. Köhler's subject was a premature boy with bilateral agenesis of the kidneys and ureters. The cerebrum showed diffuse angiomatous dilatation of meningeal and subependymal blood vessels. The latter alteration was so marked that both lateral and the third ventricles were filled with a mass of dilated blood vessels. She interpreted her case as closely related to Sturge-Weber's disease, although there was no facial hemangioma.

Several descriptive disease entities have linked renal disease with alterations of the brain, mainly vascular. Congenital aneurysm of the cerebral vessels is relatively common with renal polycystic disease.[2,3] Cerebral tuberous sclerosis may coexist with polycystic kidneys,[4] renal liposarcoma,[5] or, more commonly, connective-tissue hamartomas of great variety.[6]

Lindau[7] emphasized the relationship of cerebellar hemangioblastoma to renal and pancreatic cystic disease. In fully developed examples Lindau's disease consists of angiomatous lesions of cerebellum, retina, and spinal cord; cysts of kidneys and pancreas, and hypernephromas.[8] Lindau considered the disease as a generalized dysplasia of mesoderm. Vascular lesions of the cerebrum similar to those occurring in the cerebellum have not been reported in Lindau's disease.[9]

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Report of Case

The mother of this infant was secundigravida and primipara, who denied any personal or family history of cutaneous vascular nevi, neurofibroma, epilepsy, or mental or kidney disease. The patient was a premature boy stillborn after a breech labor preceded by an uneventful gestation of eight months. The attending physician noted a normal amount of amniotic fluid.

Postmortem Examination.—The subject weighed 2000 gm. and measured 39 cm. in length. The face presented a distinctly unusual appearance. The eyelids were somewhat edematous, with prominent epicanthic folds. The eyes were widely spaced. The base of the nose was broad and recessed; the tip was thicker than usual. The cranium appeared smaller than normal, and the anterior fontanelle was very small. The lower jaw was recessed slightly. The left pinna was large, was thrice folded, and felt soft. The left ear was lower than the right. The right ear was normal. The scrotum was empty. The anus was represented by a small dimple which could not be probed. Bilateral talipes equinovarus deformities were present. A careful search of the skin for pigmented areas or vascular nevi disclosed none.

The lungs weighed 21 gm. together and were collapsed. Microscopical examination disclosed congestion, focal intra-alveolar hemorrhage, and atelectasis. The degree of development appeared consistent with the weight and gestation period of the subject.

The heart weighed 10 gm. There was a 2×3 mm. oval defect at the base of the interventricular septum. The foramen ovale and ductus arteriosus were patent. A 4×3×2 mm. accessory spleen was present in the lienal-colic ligament. The liver was grossly normal; microscopical examination disclosed slight fatty metamorphosis of liver cells. The pancreas was normal grossly and upon microscopical examination. Both kidneys, ureters,

and renal arteries were absent. Numerous histologic sections through the fat, connective tissue, and psoas-quadratus lumborum muscles revealed no evidence of nephric structures. The bladder was 11 mm. long, with no identifiable trigone. The testes were retroperitoneal above the internal ring. A vas deferens could be traced from the testis to the base of the bladder on each side. The epididymides were not identified, and microscopically a dense mass of dilated, thin-walled capillaries largely replaced them and extended into the hilum of each testis.

The adrenal glands weighed 9 gm. together. Instead of the normal triangular configuration, the adrenals assumed flattened disk shapes. On microscopical examination they presented no abnormalities.

No abnormalities were detected in the bony structure of the ribs or the spinal column from C-3 to the coccyx. There was no spina bifida. The spinal cord was removed and presented a normal gross appearance when it was sectioned after fixation.

When the scalp was reflected, a small amount of subcutaneous edema fluid was released. The subcutaneous tissue and bones of the skull exhibited no vascular alterations. The dura mater was unremarkable. The brain weighed 105 gm. The cerebral hemispheres were covered by a dense mesh of tangled, tortuous, engorged pia-arachnoid blood vessels (Fig. 1). These varied from minute caliber up to 5 mm. in diameter. The vessels appeared to grow into the cerebral cortical tissue between the gyri and could not be lifted away. Although both cerebral hemispheres were involved, the vascular layer appeared thicker over the left lateral cerebral hemisphere from the frontal to occipital region. The base of the brain was spared; the arteries of the circle of Willis were thin, pliable, and grossly unremarkable. The pia-arachnoid of the cerebellum, the cerebellar tentorial membranes, and the large venous sinuses of the dura mater were normal. The eyes were not removed.

After formalin fixation, multiple coronal sections of the brain were studied. The left cerebral hemisphere was much smaller than the right. In areas where the number of tortuous meningeal blood vessels was greatest, the cortex was poorly formed. There were numerous empty spaces in the gray matter between the blood vessels; this gave a honeycomb appearance to the junction of the cerebral cortex and arachnoid membranes (Figs. 3, 4, and 5). The frontal-parietal and occipital lobes were maximally involved; relative sparing of the temporal lobes was noted. The vascular alterations involved the outer one-third of brain tissue; the inner centrum ovale and the basal ganglia were not remarkable. On sectioning the occipital lobes, minute flecks of firm white-gray mineralization were embedded in the white matter near dilated blood vessels; these produced a gritty sensation against the knife (Fig. 2).

The ventricular system was slightly dilated. The ependymal lining and the choroid plexus were grossly normal without vascular malformation. Sections of the cerebellum, midbrain, and spinal cord were normal.

Microscopic Findings: Coronal sections of the cerebral hemispheres were embedded in celloidin. Blocks from major areas were paraffin-embedded and stained with hematoxylin and eosin, Van Gieson connective tissue stain, Nissl stain, von Kóssa method for calcium, Prussian blue technique for iron, and Gomori reticulum stain. Frozen

Fig. 1.—Left occipital cortex. A thick ramifying network of dilated blood vessels covers the cortex.

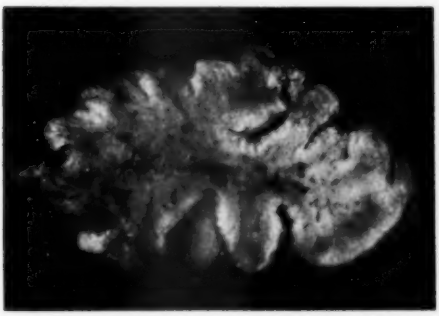
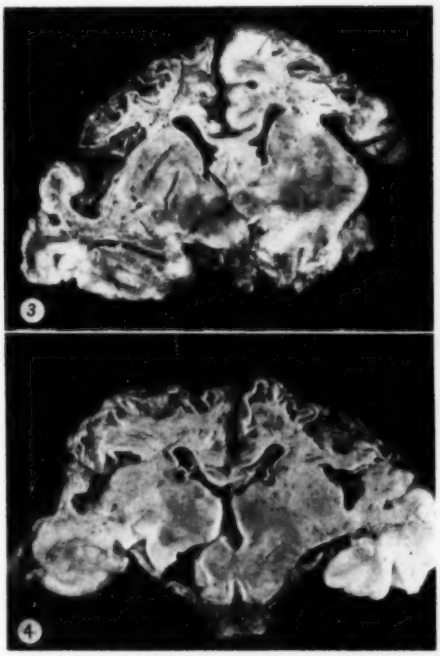


Fig. 2.—Cut surface of left occipital pole. Discrete mineral concrements in the central white matter.



Figs. 3 and 4.—Coronal sections of frontal-parietal region of brain. Distortion of gyri and honey-combed appearance of surface due to increased number and size of blood vessels, with tissue destruction.

sections of formalin-fixed material were stained for fat with scarlet red.

The vessels of the pia-arachnoid formed a thick network which distended the subarachnoid space and grew into the cerebral tissue. In general, the vessels resembled large dilated capillaries with thin connective tissue walls which lacked smooth muscle. In some, a thin elastic tissue layer was identified. The vessels tended to assume grotesque, tortuous forms (Figs. 5 and 6). This was most marked in the left parietal region. In areas of most extensive vascular proliferation the cortical gray matter was largely destroyed, having been replaced by organizing glial connective tissue. In particular, the left frontal and parietal lobes revealed almost complete obliteration of the gyral pattern (Figs. 3, 4, and 5). There was a tendency for the capillary formations to be centralized in the remnants of sulci.

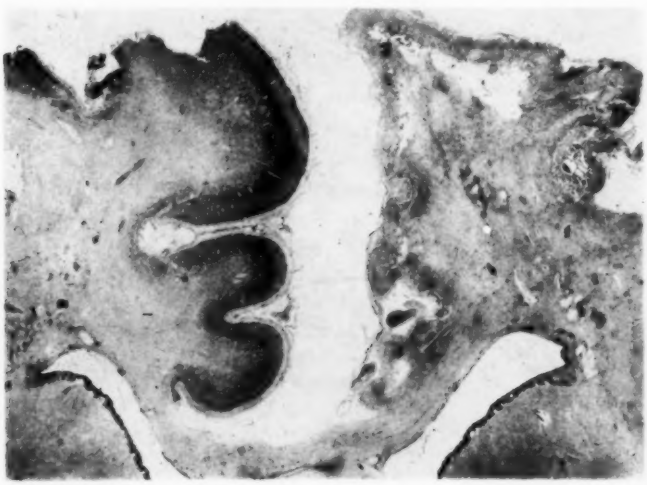


Fig. 5.—Celloidin-embedded section of central gyri and corpus callosum at same level as Figure 3. The left gray mantle is largely destroyed; the gyral pattern of the right side is abnormal. The corpus callosum is thin and contains a small cavity lined by ependyma. Reduced about 10% from mag. × 3.6.

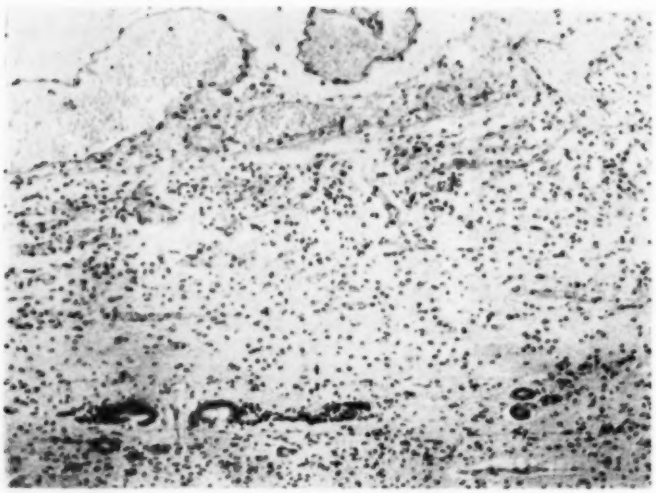


Fig. 6.—Left parietal cortex. Large thin-walled blood vessels. Extensive destruction of gray matter, with glial replacement. Small irregular ependymal islands in the white matter. Reduced about 10% from mag. × 40.

A differentiation between the abnormally vascularized tissue of the subarachnoid space and the subjacent cortical gray matter was often not possible because of a poorly defined zone of glial proliferation with focal necrosis at the depths of sulci (Figs. 5 and 6).

Various types of cortical alterations and destruction were present. Perivascular necrosis of gray matter was a prominent finding, and many blood vessels were surrounded by a cuff of fat-laden "gitter cells."

Where loss of tissue was advanced, there were small empty spaces lined by a thin glial connective tissue membrane (Fig. 5). Pigment-bearing macrophages were generally absent.

Under low magnification it was possible to trace numerous dilated giant capillaries coursing irregularly down into the cortex from the subarachnoid space (Figs. 5 and 6). Although serial sections were not performed, these vessels appeared to describe a skein-like pattern throughout the gray matter and to extend into the centrum ovale (Fig. 6). The vessels appeared closely related to the presence of tissue necrosis. In the subarachnoid and cortical gray tissue,

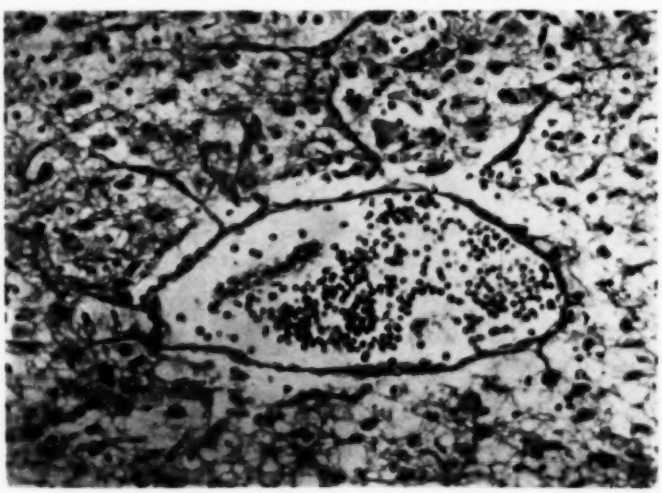


Fig. 7.—Central cortex. Perivascular tissue destruction. Gomori reticulum-Nissl stain; reduced about 10% from mag. × 320.

they were congested, widely dilated, and free of thrombi or endothelial reaction. However, in the white matter of the brain, many were occluded by thrombi which were organized to the capillary endothelium. Nearby there were numerous dilated giant capillaries which gaped open, empty of blood; this finding suggested that they were distal to a point of thrombotic occlusion.

A better insight into the vascular pattern was gained from sections stained by the Gomori silver method for reticulum fibers. These revealed an impressive increase in the number of small partly collapsed capillary structures in the gray and white matter, particularly the latter (Fig. 7). These small capillaries, inconspicuous in the hematoxylin-and-eosin-stained preparations, possessed thin walls and lumina sufficient for only two or three red blood cells. They arose in radial fashion from the dilated prominent vascular channels, which were assuming a precapillary function in this instance.

A few small ependymal nests were present in the peripheral white matter of the left parietal cortex (Fig. 6). Here the overlying gray matter was largely destroyed and replaced by glial connective tissue. Intracellular and extracellular mineral concrements were noted at the borders of necrotic tissue. These had no uniformity of size or shape, varying from 4μ to 25μ. No crystalline structure was discernible, and they were not birefringent. In the hematoxylin-and-eosin stained sections, these appeared intensely blue-black, with fuzzy outlines which merged into the surrounding tissue. The von Kóssa method for calcium and the Prussian blue stain for iron were both positive. The ferruginous concretions maintained no definite relationship to blood vessels. Within areas of necrosis in the white matter, uniformly related to abnormal blood vessels, there were individual glial cells showing ferrugination, i. e., intracytoplasmic deposition of material staining positively for iron and negatively for calcium.

Not all of the cerebral cortex exhibited the same degree of tissue destruction. In the right parietal and both temporal lobes, many areas without appreciable alteration could be found. Even in areas of relative normality there was poor definition of the layered cortical architecture, which was consistent with the immature state of the subject. Again, it is emphasized that all areas of the cerebral meninges exhibited dilated, tortuous, congested capillaries to some extent; although major pathologic alterations of cortical tissue were present only in areas of vascular penetration. The basal ganglia showed no histologic alterations.

The capillaries of the choroid plexus were moderately dilated and congested; none were thrombosed. In general, the plexus was slightly enlarged. Accumulations of neuroblasts were identified beneath the ependymal layer of both lateral ventricles. The ependymal lining of the fourth ventricle was not smooth, tending to assume an irregular serrated configuration, owing to some dilatation of subependymal blood vessels. The subependymal vessels were normal, apart from congestion.

The subarachnoid vessels of the cerebellum were slightly congested and dilated. Pathologic alterations in the cerebellum were not identified. The medulla oblongata was essentially normal, although much of the pia of this area was lost in preparation of sections.

At several levels of the spinal cord the capillaries were dilated and somewhat tortuous. There was no abnormality of the nervous tissue of the spinal cord, apart from slight gliosis. The central canal was not reduplicated or dilated.

Comment

A destructive angiomatous lesion involving virtually the entire cerebral cortex has been reported in the newborn once previously.[1] In our case a fundamental dysplasia of vascular mesenchyme in the cephalic region clearly existed; the finding of advanced gliosis with mineralization is indicative of long-standing progression in utero. The pathologic features are basically

similar to changes in Sturge-Weber's disease.[10] This well-known pathologic complex features a meningocerebral vascular lesion with a facial vascular nevus, usually within the area served by the corresponding trigeminal nerve. The patient commonly is mentally retarded and suffers contralateral epileptic seizures or hemiplegia. Roentgenographic examination of the skull may demonstrate sinuous shadows in the area of affected cortex [11]; the pathologic counterpart of this is a variable degree of mineralization of the cortex along the course of abnormal blood vessels.[12] Several recent reports [10,12,13] have clearly indicated the marked cortical destruction to be found in established cases of Sturge-Weber's complex. Notwithstanding the fact that our case lacked a facial vascular lesion, the meningocerebral vascular malformations appeared very similar to the vascular changes in Sturge-Weber's disease.

Yakovlev and Guthrie [14] have presented evidence that the vascular changes in the skin and meninges in Sturge-Weber's disease are proliferative sequelae to a basic neural abnormality related to neurofibromatosis, tuberous sclerosis, and Lindau's disease. The coordination of the latter two diseases with renal abnormalities has previously been noted. Meningeal angiomatosis has been reported in von Recklinghausen's neurofibromatosis.[15] More recently, Wohlwill and Yakovlev have extended this concept to embrace a coordinated malformation of nervous and meningeal vascular tissue with vascular hyperplasia, possibly resulting from a defect in vasomotor innervation in the involved areas. These authors, and others,[13,16] have noted visceral angiomatous lesions in lung, stomach, and small and large bowel occurring in subjects with Sturge-Weber's complex; they did not report renal abnormalities.

The anomalous meningocerebral blood vessels consist of dilated capillaries and prevenules with a single endothelial layer and connective tissue wall largely devoid of smooth muscle. Bergstrand, Olivecrona, and Tönnis [17] stated that the malformations of Sturge-Weber's complex lie between telangiectasis and racemose hemangioma. The former term, sanctified by long usage in the European literature, implies dilatation of preformed capillaries, itself a controversial histogenetic opinion. Our studies showed no evidence of arteriovenous communication. The vascular lesions in this case, as well as those of Sturge-Weber's complex, are truly hamartomatous malformations. The vessels appear to have slowly expanded in longitudinal and circumferential dimension at the expense of surrounding cerebral tissue. Whether this constitutes growth in the strict sense of the word or is a passive enlargement of preformed anomalous capillaries in response to circulatory pressure is not definite, although our material tends to emphasize the former process.

Potter [18] described three newborn infants with diffuse increase in the number of arachnoidal capillaries and prevenules. The process was symmetrical, involving both cerebral and cerebellar hemispheres. The underlying cortical tissue was normal. In all three of her cases cardiac abnormalities were present; two hearts were diffusely hypertrophied, and one infant had persistence of the left innominate vein. The marked destruction of cerebral tissue and the dramatic sparing of the cerebellum sharply delineate the difference between the case reported here and Potter's diffuse angiectasis.

The peculiar facial configuration frequently noted in subjects with bilateral renal agenesis [19-21] suggests to us that this condition is often accompanied by coordinated mesenchymal maldevelopment of the cephalic region. In a typical example, the infant appears senile; there is increased space between the eyes, with a prominent inner canthic fold and recessed nasal root; the ears are enlarged, poorly chondrified, and set rather low on the skull, and the chin is recessed. The presence of these features in our case permitted the diagnosis of renal agenesis to be suggested before

commencing the autopsy. Severe renal hypoplasia or cystic disease may give the same facies.[6,22]

In considering the etiology of the characteristic facies of renal agenesis, we have noted that rather similar facial abnormalities may occur in various types of cephalic mesenchymal dysplasias which are unaccompanied by renal disease, namely leprechaunism, mandibulofacial dysostosis, and hypertelorism. These entities, which are by no means well defined, may share with the present subject such features as widely spaced eyes with broad flat nasal root, low-set malformed ears, and small mandible. Hypertelorism is basically a result of abnormality in the sphenoid bone, with overgrowth of the lesser wings and comparative smallness of the greater wings.[23] Leprechaunism[24] is an endocrinopathy featuring multiple ovarian cysts, mammary hypertrophy, and excess glycogen and iron deposition in the liver. The syndrome occurs in undernourished underdeveloped infant girls with large low-set dechondrified ears, widely spaced eyes, and other facial features common to the facies of renal agenesis. Renal abnormalities are not reported in leprechaunism. The basic features of the syndrome are believed to result from excess estrogen production. McKenzie and Craig[25] have discussed the role of abnormal vascular patterns in the developing first and second visceral arches in producing forms of mandibulofacial dysostosis. Their careful dissection showed that a morphologic variation in the stapedial artery is probably basic, with secondary changes in the external carotid system and subsequent malformations of facial bones. Since we were not then cognizant of the work of McKenzie and Craig, facial dissection was not performed in our subject.

The presence of the facies of renal agenesis in sirenoid monsters has been noted by Fritsche.[26] He was able to study 83 examples of sirenoids and found the characteristic facial changes in 17 and suggestive changes in 8 additional. He concluded that this represented a coordinated maldevelopment of the skull which was linked to the axial skeletal abnormalities.

The link between developmental abnormalities of the axial skeleton and the meningocerebrum is the core of the problem presented by this case. Ostertag[27,28] has described cases with developmental abnormalities of the urinary tract and spine which exhibited various types of coordinated cerebral abnormalities, i. e., vascular hyperplasia of choroid plexus and connective tissue, abnormal patterns of the cerebral gyri, periventricular gliosis, and hydrocephalus. Ostertag and other European authors[21,26] have ascribed a basic role to the process of closure of the neural canal. Renal anomalies are considered by these authors to arise in consequence of abnormal growth of the dorsolateral wall of the neural canal (i. e., the alar plate), which exerts an effect upon the lateral nephrogenic mesenchyme. Since the telencephalic primordium of the cerebrum is formed from the rostral portion of the alar plate,[29] Ostertag has categorized the various cerebral abnormalities mentioned above as "dysraphic equivalents," in the sense that they result from the basic abnormality in closure of the dorsal raphe of the rostral neural canal in a manner similar to the renal abnormalities consequent upon dysraphia at the lumbar level.

The final phase of closure of the rostral neural canal, the sealing of the anterior neuropore, may affect the vascular pattern of the head and the configuration of the face. The triangular area, the anlage of the central face,[29] lies at one point near the anterior neuropore.[30] Closure of the neuropore is a relatively protracted process initiated at 13 somites and extending to 20 somites. The sealing of neural ectoderm from amniotic fluid is accompanied by a compensatory growth of new capillaries which begin to invest the neural tube.[31]

Meningocerebral vascular abnormalities such as occurred in this case and in Sturge-Weber's disease may be traced subsequent

to the period which Streeter[32] indicated in the 13 mm. human embryo. He described the primitive vascular network which supplies in common the integument and brain of the embryo under 10 mm. The developing cranium produces a lamellar cleavage so that three separate circulatory systems are formed: the superficial cutaneous, dural, and arachnoid-cerebral plexuses. Vascular anomalies affecting one of these circulatory beds and sparing the others may be expected to arise after the lamellar separation.

Renal agenesis has an earlier embryonic basis, having been established before the 13 mm. stage at the seventh week of embryonic life. Previous authors[20,33,34] have stressed the role of the ureteric buds in failing to organize the metanephric mesenchyme either through faulty differentiation of the lower mesonephric duct or lack of adequate physical contact between the advancing ureteric duct and the metanephric cap.[22]

The findings in our own case, however, suggest that renal agenesis may occur as part of a continuing widespread tissue disorder which is manifested by coordinated neuromesenchymal defects far removed from the urogenital ridges. The basis for the abnormalities was established in the growth period from 5 to 25 mm., commencing with early renal differentiation and embracing the period of closure of the rostral neuropore and meningovascular development.

We are unable to indicate a single basic intrinsic or extrinsic noxious agent which may have produced this remarkable constellation of abnormalities. Perhaps the critical factor is the time and degree of tissue damage or arrest rather than the means by which it is affected. Our case does not support Ostertag's thesis that renal and cerebral abnormalities are subordinate to closure of the neural canal. The abnormalities of renal and facial development and meningovascular angiomatosis are closely coordinated, but there is no convincing evidence of the primacy of one organ system.

Summary

The concurrence of bilateral meningocerebral angiomatosis with renal agenesis is reported, and the similarity of these meningeal alterations to Sturge-Weber's disease is noted. The coexistence of coordinated defects in the development of the head and urogenital system is emphasized. The embryologic basis for meningocerebral angiomatosis, renal agenesis, and facial alterations is reviewed, and the conclusion is advanced that these are the result of a widespread neuromesenchymal tissue defect.

Prof. W. Scholz and Prof. H. Burkhardt gave valued advice in this study.

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Atherosclerosis in Rhesus Monkeys

I. Hypercholesteremia Induced by Dietary Fat and Cholesterol

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Introduction

The multiplicity and complexity of factors involved in the genesis and progression of atheroarteriosclerosis have stimulated extensive experimental studies. In many of these studies it has not been feasible to use human subjects. Since the initial experimental production of diet-induced atheromata in rabbits in 1908,[1] various other species of animals have been studied, including the hamster,[2] guinea pig,[3] dog,[4,5] rat,[6-8] chicken,[9] parrot,[10] parakeet,[11] cat,[12,13] goose,[14] duck,[15] pig,[16] goat,[17] cow,[18] and monkey.[19-22] Certain similarities, as well as many dissimilarities, may be pointed out between several of these species and man.

Our laboratory accepted the monkey as possessing more similarities to man than any of the other species mentioned. Similarities between the monkey (Macaca mulatta, popularly known as rhesus) and man include anatomy of the body and vascular system[23]; blood types[24,25]; relative duration of periods of life, i. e., a maximal life expectancy of about 25 years with sexual maturity at 4 to 6 years[26]; menstrual cycle; period of gestation and pregnancy[27]; requirements for nutritional factors,[28] and susceptibility to many microbiological diseases.[29-31] Our current studies suggest that there also exist certain similarities between man and monkey in their metabolism of fats, including cholesterol.

Earlier studies reported generally negative results from efforts to induce significant hypercholesteremia in monkeys with a diet enriched in cholesterol and total fat. Recent studies have described the induction of hypercholesteremia with diets providing high levels of total fat and cholesterol but including deficiency of some essential nutritional factors. In their report, Mann, Andrus, McNally, and Stare[32] emphasized the necessity of a dietary deficiency of sulfur amino acids, in addition to a high level of total fat and cholesterol, for the production of hypercholesteremia in Cebus monkeys. On this regimen the animals showed marked elevations of serum cholesterol, with fairly extensive vascular atheromatosis, in spite of progressive weight loss. A more recent publication[33] from the same laboratory reported marked hypercholesteremia with florid atheromatosis and skin xanthomatosis in a pet monkey eating a diet very high in cholesterol and supplying 70% of total calories in the form of fat. A sulfur amino acid deficiency was apparently not essential to the hypercholesteremia in this animal. Studies by Rinehart and Greenberg[34,35] demonstrated an effect of pyridoxine deficiency in the production of experimental vascular lesions in monkeys. These lesions were described as re-

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sembling early arteriosclerotic lesions in humans.

To the best of our knowledge these dietary modifications do not closely simulate human dietary conditions. The evidence for a generalized dietary deficiency of pyridoxine is meager. As for sulfur amino acid deficiency, Mann et al. did not claim that this artificial diet has any counterpart in the human diet. Certainly, it is contrary to the general clinical experience of internists and pathologists to find accentuation of hypercholesteremia and atheromatosis associated with prolonged progressive weight loss,[36,37] as was the case with the monkeys of Mann et al. In addition, our personal experience is that most investigators will have considerable difficulty in obtaining monkeys with an actual preference for a high-fat diet, as reported for the single pet monkey mentioned above. For these reasons, we feel that our observations may be of value to other investigators who wish to study experimental vascular disease in an animal as similar to the human as possible.

This paper reports data and conclusions from part of our studies on the response of rhesus monkeys to experimental diets enriched in cholesterol and total fat. Techniques for the care and handling of monkeys in laboratory studies have been discussed elsewhere.[38-40] Our diets and methods of feeding will be discussed in detail in this paper because of the importance and difficulty of presenting dietary fat in a form that monkeys regard as palatable. Initially, we posed several questions: 1. Will the monkey eat a high-fat, high-cholesterol diet? 2. Will the monkey permit or afford frequent blood samples of sufficient quantity for determination of cholesterol, lipid phosphorus, total lipid, and ultracentrifuge patterns? 3. Will it give a hypercholesteremic response to a diet which contains adequate amounts of the known essential nutritional factors but is high in cholesterol and total fat? 4. Can vascular atheromata be induced in monkeys under conditions which, with some qualifications, simulate human conditions? Preliminary reports from our laboratory have given an affirmative answer to each of these questions.[41-44]

Materials and Methods

To date, more than 30 animals have been studied for periods varying from several months up to three years. Many of the monkeys were obtained from Okatie Farms, Pritchardville, S. C., and some from S. C. Somerville, Savannah, Ga. Most animals exceeded 8 lb. (3.6 kg.) at the time of purchase. Their ages were not known, but we attempted estimations of their ages by the characteristics of dentition, body size and weight, and menstruation in the females.[45] Table 1 tabulates certain basic data for 30 animals.

With our more recent diets the general health of the animals has been excellent. Throughout the study some morbidity and mortality have occurred from parasitic infestation of the lungs and gastrointestinal tract. Recently, an outbreak of fulminating disseminated tuberculosis resulted in the loss of nine animals. Tuberculosis in rhesus monkeys poses a serious hazard to their use in laboratory studies, but precautionary measures are available.[46-49] Minimizing contact between individual animals, periodic cutaneous tuberculin sensitivity tests, and x-rays of the chest have been of definite value.

New animals were kept on a stock, low-fat diet for several months until the serum cholesterol values became relatively stabilized. After the institution of the experimental high-fat, high-cholesterol, diet, blood samples were drawn at intervals of two weeks originally and four to six weeks more recently. During part of the study, each animal donated approximately 40 to 50 ml. of blood every other month and 10 ml. on alternate months; the larger sample provided ample serum for all of our own determinations plus ultracentrifuge studies at the Cleveland Clinic. Gross anemia was never observed when blood samples were spun down for the separation of serum from cells.

Blood samples were taken from the femoral artery or vein, with use of a No. 20 needle, with the animals rigidly restrained on a heavy V-shaped platform. Usually, these were taken 12 to 24 hours after a meal. Catching and restraining the animals always disturbed them greatly, as manifested by the transient autonomic responses of urinary and fecal incontinence. Although many animals eventually became relatively tame, none ever made a completely peaceful adjustment to the process of being restrained for venipuncture.

Serum total cholesterol determinations were performed initially according to the method of Abell

TABLE 1.—*Pertinent General Data on Monkeys Reported in this Study* *

Animal	Sex	Age (Est.) at Time of Purchase, Years	Weight, Kg.				General Health	Mode of Death
			Initial	Minimal	Maximal	Terminal		
Dorothy †	F	5					Fair	Autopsied for study
Mary †	F	6					Good	Autopsied for study
Smiley	F	9	5.1	3.7	5.2	5.0	Good	Autopsied for study
Betty ‡	F	4	4.1	3.7	5.8	5.6	Excellent	
Irma	F	4 3/4	4.2	4.2	4.7	4.2	Excellent	Autopsied for study
Joe	M	5	7.0	5.5	7.0	6.9	Fair-poor	Killed for chronic illness
Bruce	M	5 1/2	5.4	4.4	5.4	4.7	Fair-poor	Killed for chronic illness
Henry	M	4 3/4	4.5	4.5	8.5	8.5	Excellent	Surgical anesthetic death
Olive ‡	F	4	4.9	3.8	5.4	5.0	Excellent	
Henrietta	F	3 1/2	5.2	3.8	5.3	3.8	Poor	Killed for illness
Josephine	F	5	5.7	4.1	7.5	6.8	Excellent	Asphyxiation during tube feeding in balance study
Clark	M	3 1/2	4.3	3.0	4.3	3.0	Poor	Autopsied for study
Oliver	M	4 1/2	4.5	3.9	4.7	4.7	Fair	Postoperative aortic thrombosis
Horace	M	4 1/2	5.3	5.3	7.4	6.9	Excellent	Autopsied for study
Archy	M	5	5.9	3.4	5.9	3.4	Poor	Died in course of therapy for gastrointestinal parasites
Sylvester	M	3 1/2	5.3	5.0	7.1	7.0	Excellent except terminally	Killed for pulmonary TB
Herman	M	5 1/2	6.8	6.1	7.4	7.1	Good	Killed for pulmonary TB
Jack	M	3 1/2	5.4	4.7	6.8	5.7	Fair	Died from TB
Jim	M	3 1/2	6.1			6.1		Shock following surgery
Pete	M	5	5.7	5.4	7.2		Excellent except terminally	Died from TB
Yvonne	F	4	4.0	4.0	5.1	4.0	Good-excellent	Killed for TB contact
Jett ‡	F	3 1/2	3.8	3.8	4.8	5.1	Excellent	
Jill	F	5 1/2	5.3	4.7	8.1	8.1	Excellent	Surgical anesthetic death
Emma	F	5 1/2	3.2	3.2	4.7	4.7	Good-excellent	Killed for TB contact
Nora	F	5	4.5			4.5		Shock following surgery
Sheba	F	3 3/4	3.7	3.0	4.5	3.0	Poor	Killed for TB
Missy	F	9	5.7	5.4	8.2	7.9	Excellent	Killed for TB contact
Gertrude	F	12	5.2	6.9	6.6	5.9	Fair	Died from TB
Kett	F	9	5.7	4.8	6.0	4.8	Fair-poor	Died from TB, protracted course
Stella ‡	F	3 3/5	6.2	6.2	7.2	7.1	Excellent	

* Animals arranged in order of purchase and study.
† These animals comprised an initial pilot experiment; complete data were not obtained.
‡ These animals are still living in excellent health, with slow steady weight gain; thus we do not have terminal (meaning at death or autopsy) values for them.

et al.[60] Later the Sperry-Webb modification of the Schoenheimer-Sperry technique was employed for determination of serum total and free cholesterol.[61] Lipid phosphorus was determined by the method of Youngberg, modified by application of the phosphate procedure of Fiske and Subbarow.[62] Phospholipid was calculated by multiplying the lipid P concentration in milligrams per cent times a factor of 25. Initially blood fatty acids were determined by the method of Stoddard and Drury[63]; later, this determination was replaced by the Kunkel technique for serum total lipids.[64] For ultracentrifuge patterns, aliquots of serum were prepared, cooled, packed into precooled thermos bottles, and shiped via air express to Dr. Lena Lewis at Cleveland Clinic, Cleveland.

Most animals were subjected to one or more abdominal operations for the twofold purpose of (1) obtaining liver, intestine, and skin biopsy specimens for in vitro estimation of endogenous cholesterol synthesis[65,66] and (2) producing segmental arterial lesions by freezing small discrete areas in the major abdominal arteries.[42,43] Subsequent operations permitted biopsy and histologic study of previously injured vessels, generally without any apparent impairment in the animal's health or physiology. Several animals have each survived five or more abdominal operations over a period of two or more years with no evidence of complications or permanent injury to their general health. Standard sterile surgical procedures were employed. One dose of procaine penicillin G (300,000 units) was usually administered postoperatively. Pentobarbital (Nembutal) (30 mg. per kilogram) either intraperitoneally or intravenously provided satisfactory anesthesia for two to four hours. The safety margin of this anesthetic for monkeys appears smaller than for dogs. Inefficient administration with some of the original dose going into subcutaneous tissues, followed by an additional small dose to hasten anesthesia, was attended by death in two cases. Two deaths apparently were due to irreversible shock following excessive surgery that required four to five hours' operative time. Most animals withstood surgery exceedingly well if the operative time did not exceed about three hours; but all showed loss of appetite and weight for the first three to four postoperative days and sometimes longer.

Diets

Stock Diet No. 1 (Low Fat, No Cholesterol).—Initially, each animal was fed twice daily. The morning ration consisted of two slices of dry white bread soaked in skimmed milk made from powdered skimmed milk and water. The afternoon ration was varied, usually consisting of any three of the

following: apples, oranges, sweet potatoes, carrots, prunes, lettuce, and cabbage. Occasionally various fruit flavors and/or sugar were added to the bread and skimmed milk in an effort to increase its palatability. This provided a diet low in fat and essentially free of cholesterol; however, no bread-and-milk formula was ever found that was eaten well by the monkeys. The fruits were always eaten well.

Stock Diet No. 2 (Low Fat, Low Cholesterol).—This is the stock diet currently being used in our studies. It is a ground monkey-food "mash" to which water is added to make a thick, uniform "porridge." This food is a commercial preparation,* with the ingredients listed as wheat flour, wheat germ, soy bean oil meal, dried skimmed milk, alfalfa-leaf meal, sucrose, bone meal, and vitamin mix. In recent correspondence from the company, complete analyses were stated as follows:

Ingredient	Amount
Protein	19.1%
Fat	2.1%
Fiber	2.8%
Ash	3.95%
Nitrogen-free extract	12.2%
Calcium	0.4%
Phosphorus	0.7%
Arginine	0.8%
Lysine	0.7%
Methionine	0.3%
Cystine	0.2%
Tryptophan	0.1%
Glycine	1.5%
Iron	0.001%
Manganese	6.8 mg./lb.
Copper	1.6 mg/lb.
Cobalt	0.37 mg/lb.
Vitamin A	7610 U. S. P. units/lb.
Vitamin D	2996 U. S. P. units/lb.
Riboflavin	1.1 mg/lb.
Nicotinic acid (niacin)	24.0 mg/lb.
Pantothenic acid	6.4 mg/lb.
Choline	593.0 mg/lb.
Total calories	812/lb.

The dry food is easily stored, and fresh portions are taken daily to which is added the required amount of water (hot water can be used to make "hot porridge") plus extra supplements of vitamin K, ascorbic acid (vitamin C), folic acid, and pyridoxine. Fruit supplements were not given, except two or three prunes daily as "treats" for good behavior and for supplemental iron, especially after large blood samples were taken. This diet has been eaten well by all that received it. For convenience, if desired, larger quantities can be made and stored at 4 C from which portions can be taken daily. The food will keep for several weeks in this form, and most animals after a short period of adjustment eat the "cold porridge" about as well as the hot.

Cholesterol Diet No. 1.—To the Stock Diet No. 1 was added daily supplements of 3 gm. of cholesterol† and 15 gm. of vegetable oil (corn oil). The cholesterol was dissolved in acetone, then soaked into slices of dry white bread. Evaporation of the acetone left the bread impregnated and coated with cholesterol, supplying an average of approximately 3 gm. of cholesterol per daily feeding of two slices of bread per monkey. The vegetable oil was homogenized with the skimmed milk in a Waring Blendor or poured directly onto the cholesterol-impregnated bread, which was then broken up in the skimmed milk. This provided a reasonably standard diet which contained very little extraneous unknown fat; however, it was eaten poorly by most monkeys. Fruit flavors and/or sugar improved its apparent palatability very little. The afternoon meal consisted of fruits as in Stock Diet No. 1.

Cholesterol Diet No. 2.—Cholesterol-saturated bread was used as in Cholesterol Diet No. 1, supplying 3 gm. of cholesterol per monkey per day. Cow's cream (15% butterfat) was substituted for the skim milk-vegetable oil suspension; 100 ml. of cream per animal per day supplied nearly the same quantity of fat as Cholesterol Diet No. 1. Cream was tried because of an earlier observation of hypercholesteremia in a single monkey (Mary) ingesting a cream-rich, high-cholesterol diet as part of a separate study. In general, this diet was as unsavory to the monkeys as the previous high-fat, high-cholesterol diet.

Cholesterol Diet No. 3.—Daily portions of 3 gm. of cholesterol and 15 gm. of butter per monkey were mixed with freshly cooked rice and served as a thick warm preparation. Fruits comprised the afternoon meal. In spite of the mouth-watering effect of this diet on some of the project members, it was eaten with little real enthusiasm by the monkeys, and it had certain difficulties in its preparation which led to its early abandonment.

Cholesterol Diet No. 4.—Daily portions of 3 gm. of cholesterol and 30 gm. of butter per monkey were added to about 175 gm. of ground monkey food with vitamin supplements, as in Stock Diet No. 2. The dry powdered cholesterol was stirred into the dry mash; the butter was homogenized with hot water in a Waring Blendor and added to the cholesterol-enriched mash to make a thick "porridge." Fruit supplements were not given, beyond occasional prunes and infrequent pieces of cabbage. This diet can also be made up in large bulk and stored in the refrigerator for daily rationing as Stock Diet No. 2. However, the presence of the

* Dietrich and Gambill, Inc., Fredrick, Md.

† The cholesterol was supplied by Armour and Co., Chicago.

TABLE 2.—*Estimated Daily Intake of Carbohydrate, Protein, Fat, and Cholesterol* *

Dietary Regimen	Carbohydrate		Protein		Fat Other Than Cholesterol		Cholesterol	
	Gm.	% Total Cal.	Gm.	% Total Cal.	Gm.	% Total Cal.	Gm.	% Total Gm.
Stock diet No. 1	57	77	13	17	2	6	0	0
Stock diet No. 2	56	75	12	16	3	9	Trace	
Cholesterol diet No. 1	40	54	12	16	10	30	1.5	2.4
Cholesterol diet No. 2	40	54	12	16	10	30	1.5	2.4
Cholesterol diet No. 3	44	59	8	11	10	30	1.5	2.4
Cholesterol diet No. 4	32	43	11	15	14	42	1.5	2.6
Cholesterol diet No. 5	32	43	11	15	14	42	1.5	2.6

* The total food intake in grams exceeded the sum of the figures given for carbohydrate, protein, and fat; the difference consisted of fiber and moisture; 300 Cal. per day is taken as an approximation of the average daily ingestion of the animals reported herein. Variation in grams of total food intake were apparent when different diets were used; even greater variation was noted between individual animals on the same diet. In addition, measurements of food intake were exceedingly difficult and frequently unreliable because of the wasteful eating habits of the monkeys. The above figures, therefore, are no more than estimations.

butter definitely requires a low temperature (approximately 4 C) to prevent rancidity. Almost all animals given this diet have eaten it very well, although some required a few days of acclimatization to its greasiness. The greasiness is less prominent if a minimal amount of water is used, just enough to make the "porridge" stick together. (Note: If the cholesterol was lumpy, a much finer dispersion of the cholesterol in the mash and butter was obtained with the use of a Waring Blendor. The cholesterol, butter, a portion of the dry mash, vitamin supplements, and hot water were placed in the Blendor and homogenized into a homogeneous suspension, then mixed with the rest of the mash, with water added as necessary.)

Cholesterol Diet No. 5.—This was essentially Cholesterol Diet No. 4 modified by adding one canned egg yolk daily. This diet was tried with only one animal, which ate it with somewhat less gusto than it later ate Cholesterol Diet No. 4.

Table 2 summarizes some of the features of the diets.

Results

General.—After two years of coaxing monkeys to eat, sometimes just to taste, various diets; after many efforts to outsmart them by "hiding" the cholesterol in the food in various ways that made it undetectable to us, and after much "seasoning" with apple sauce, fruit flavors, fruit-flavored sherbets, and other vehicles had failed to induce the animals to act interested in our high-fat diets, we became convinced that studies of dietary fats in monkeys require the fat to be presented in just the right way. Otherwise, the animals eat poorly or sometimes not at all, and the study suffers from artifactitious data. Rhesus monkeys generally prefer low-fat foods, especially fruits. Their native diet is believed to be primarily, although not entirely, vegetarian, and the embellishments of civilized cooking usually have little appeal to animals long habituated to their native diets. That monkeys in captivity will sometimes eat freshly ground horse meat [57] does not mean that they are by nature (i. e., taste and metabolism) carnivorous. Addition of fats, especially cholesterol, to their food generally invites extensive picking, smelling, careful tasting, and ultimate scattering and wasting, with little actual ingestion. In the early part of our studies the monkeys were housed in separate cages that contained sawdust on the bottoms. Several animals became extremely adept at hiding the fat meal in the sawdust and existing almost entirely on fruits and vegetables. Nearly all animals regarded our initial high-fat diets (Cholesterol Diets Nos. 1, 2, and 3) as unpalatable and subsisted on fruits and vegetables when they were available to them. Realization of this led to a further search for the "right" diet. The ground monkey food chanced to be well eaten by nearly all animals and proved to be an excellent medium for mixing in quantities of fat or other substances, without losing much of its appeal to the monkeys. After this was discovered, we adopted a one-food, one-meal technique whereby one meal of a homogeneous preparation was given per day with almost no other food or fruits being given. On this regimen (Stock Diet No. 2 for control animals and Cholesterol Diet No. 4 for experimental animals), animals ate much better and gen-

erally appeared healthier, and the experimental animals gave a much faster and greater response to dietary cholesterol than on earlier regimens. Currently, the one-food, one-meal technique, plus the use of cages with large-bore steel-mesh bottoms, provides considerable control over what the monkeys eat and permits reasonable information as to how well they eat.

The monkey's custom of testing and throwing away nearly every other handful of the fat-enriched food, which rapidly becomes mixed with excreta, completely thwarts reasonable attempts to measure their daily intake. The steel mesh cage bottoms have the one disadvantage of increasing this wasteful scattering. Our usual practice is to feed them enough to allow for this waste, which amounts to from one-third to two-thirds of the portion that they are given. We are currently attempting balance studies on several monkeys given a high-fat diet. Preliminary data (on adult female rhesus monkeys essentially at rest) indicate an average daily requirement of 275 to 325 Cal. per day per monkey for body weight of 5 to 7 kg. This averages about 45 to 55 Cal. per kilogram of body weight, which compares well with published values of 41 to 54 metabolizable calories per kilogram of body weight as minimum requirements of rhesus monkeys.[58] In Table 2, for simplicity of presentation and for lack of more detailed measurements, the average daily ingestion is taken to have a caloric value of 300 Cal.; this is approximately one-half the daily quantity actually given to each animal. The average daily ingestion of our monkeys was severalfold less than that reported for control rhesus monkeys of about 2 kg. of body weight.[59] We cannot but wonder if these reported figures included such food given to the monkeys but not actually eaten by them.

On all diets, the animals showed occasional periods of voluntary fasting. Such fasting sometimes occurred when animals were first given the high-fat diet and infrequently after long periods of apparently satisfactory adjustment to the diet. At such times it appeared that the animals were "striking" for food of their choice, for they would delightedly eat fruits while completely refusing the experimental diet. The periods of fasting occasionally extended up to one to three weeks and usually correlated with periods of sickness or surgery but sometimes occurred with no reason known to us. Surprisingly enough, such long periods of partial or complete fasting generally caused the animals no obvious loss of energy or strength, as judged by their perpetual resistance to being restrained for venipuncture.

Many animals suffered one or more periods of sickness, which usually were vague in nature, characterized by an attitude of relative inactivity, diminished appetite, occasional vomiting, or in some instances diarrhea, with loss of weight being the chief physical finding. Such sickness in a few animals continued intermittently for many weeks and was associated with either depression of the blood lipids or failure to rise if they were already low. One animal was found to have an unidentified gastrointestinal parasite, and two at autopsy showed extensive lung mites (acariasis).[60] But in many instances the "sickness" was judged to be a manifestation or result of the animal's dislike for certain high-fat diets and relief was frequently most easily obtained by offering foods that accorded to the animal's natural preference (low-fat foods, especially fruits and vegetables).

Control Serum Cholesterol Levels.—Initial blood samples were taken within four to eight weeks after arrival of new monkeys and institution of the stock diet. Some animals, primarily the first 12 purchased (the "first group" of Table 3) showed relatively low initial serum total cholesterol levels (average, 122 mg.%), which increased slightly after an interval on the stock diet (to an average of 132 mg.%). Some animals of the "second group," purchased later, had relatively high initial values (average

TABLE 3.—*Average Serum Total Cholesterol in Monkeys Eating a Control Diet* *

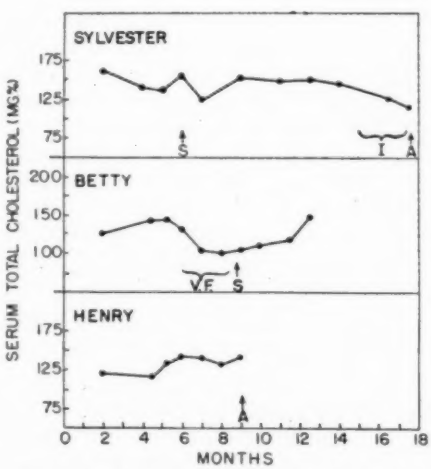
Group of Animals	Diet	Method	Animals, No.	Determinations, No.	Serum Total Cholesterol, Mg. %		
					Range	Mean	Standard Deviation
First	Largely Stock Diet No. 1	Abell et al.	12	36	92–165	132	16.8
Second	Stock Diet No. 2	Abell et al.	15	32	113–213	148	22.1
Second	Stock Diet No. 2	Sperry & Webb	16	44	116–228	153	23.2
Total of first and second	Stock Diet Nos. 1 & 2	Abell et al.	27	68	92–213	140	19.4

* Stock Diet No. 1 or 2.

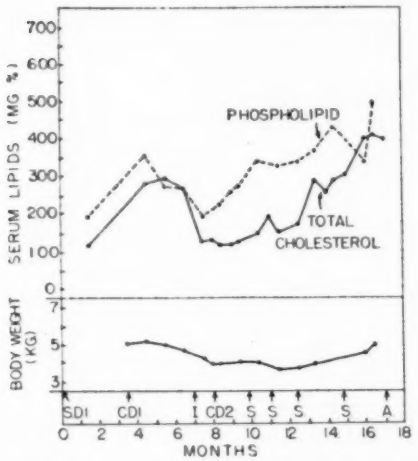
of 155 mg.%), which fell slightly on the stock diet (to an average of 148 mg.%). These values were determined by the method of Abell et al. For one and one-half years after the introduction of the Sperry-Webb technique, the total cholesterol of serum samples was determined by both it and the technique of Abell et al. The Sperry-Webb values generally exceeded the Abell values by an average of 4 mg.%. This probably resulted largely from greater care given to certain steps in the Sperry-Webb procedure. (Extra effort in taking the proper size aliquot of the alcohol-acetone extract to achieve in the color development an optical density simulating that of the standards minimized potential inaccuracies in the application of Beer's law.) Table 3 summarizes the control serum total cholesterol values, showing the variation between our first and second groups of animals (purchased approximately one and one-half years apart), the variation between methods, and the average of all control cholesterol values.

Most fluctuations in control cholesterol levels were in the direction of depressions that generally correlated with the presence of sickness, surgery, or periods of self-imposed fasting. Graph 1 shows the control cholesterol levels of two monkeys given Stock Diet No. 1 and one given Stock Diet No. 2. Henry ate Stock Diet No. 1 exceptionally well, with no marked periods of sickness or fasting, and gained weight progressively. Betty ate poorly, with some weight loss. Sylvester ate Stock Diet No. 2 very well, with progressive weight gain. Note the long low trough in Betty's curve, while Henry and Sylvester hovered much closer around a level of 140 to 150 mg.%. Henry and Betty were of the "first group" and showed low initial cholesterol levels. Sylvester's curve shows a slightly elevated initial cholesterol level, with prominent depressions associated with surgery and, terminally, tuberculosis. Table 3 shows 140 mg.% to be the average of all control serum total cholesterol determinations performed on the monkeys by the method of Abell et al. This checks very closely with the value of 142 mg.% reported by Mann et al.[32] for Cebus monkeys, but is considerably higher than the value of 118 mg.% reported elsewhere.[61]

Effect of High-Fat, High-Cholesterol Diet.—Total Cholesterol: Cholesterol Diets



Graph 1.—Control serum total cholesterol of three monkeys, showing typical depressions associated with surgery, illness, or simply distaste for the diet. *S* indicates surgery; *V. F.*, voluntary fasting, apparently resulting from dislike for the diet (Stock Diet No. 1)—no physical illness was apparent; *I*, illness (tuberculosis in the case of Sylvester); *A*, autopsy.



Graph 2.—Response of a monkey (Smiley) to cholesterol feeding, with intermittent periods of depression following illness and surgery. *S. D. 1* indicates Stock Diet No. 1; *C. D. 1*, Cholesterol Diet No. 1; *C. D. 2*, Cholesterol Diet No. 2; *I*, illness; *S*, surgery; *A*, autopsy.

Nos. 1, 2, and 3 rarely elevated the serum total cholesterol beyond 300 mg.%. Graph 2 shows the maximum response obtained with these earlier diets. This particular animal (Smiley) was subjected to considerable surgery and also had periods of sickness; her weight fluctuated up and down, but the net change was a loss. However, other animals with less surgery and sickness and some weight gain showed a similar poor response to these diets.

The introduction of Cholesterol Diet No. 4 resulted in a different response from the monkeys. The ground monkey-food "porridge" was generally very well eaten, and almost all animals given it showed progressive weight gain with good general health marked by considerably fewer and shorter periods of sickness and fasting than had been noted with earlier diets.

Our earlier discouragement with the study was completely dispelled by the rather rapid elevation of serum cholesterol to heights of three, four, or five times the average control level. In several instances, four to six weeks sufficed to attain twofold or threefold elevations of the serum cholesterol levels. Maximum cholesterol values ranged between 500 and 700 mg.%, with one peak value of 732 mg.%. Tables 4 and 5 summarize the data from serum cholesterol and phospholipid determinations. In Table 4

TABLE 4.—*A Summary of the Serum Total Cholesterol and Phospholipid Levels Induced by Several High-Fat, High-Cholesterol Diets*

		Serum Total Cholesterol					Serum Phospholipid (Lipid P×25)				
		Initial	Maximal		Final		Initial	Maximal		Final	
Animal	Cholesterol Diet No.*	Mg. %	Mg. %	Weeks Given Diet	Mg. %	Weeks Given Diet	Mg. %	Mg. %	Weeks Given Diet	Mg. %	Weeks Given Diet
Dorothy †	1	164	397	8	157	12					
	2	157	215	1	82	16					
Mary	1	154	339	12	339	12					
	2	339	572	4	300	43					
Smiley †	1	120	295	7	132	15	195	353	4	197	15
	2	132	413	38	413	38	223	493	38	493	38
Betty	2	150	249	3	215	8	293	402	3	318	7
	4	215	669	28	500	98	318	495	21	432	92
Irma ‡	1	129	153	4	136	33	203	285	27	265	33
	2	136	185	10	185	10	265	338	5	325	10
	4	185	267	44	172	48	325	340	39	280	48
Henry	2	144	276	19	276	19	263	338	19	338	19
	4	276	274	9	274	9	338	420	5	353	9
Olive	4	152	274	61	266	69	307	370	9	330	62
Josephine	4&5	142	394	91	363	97	250	411	91	411	91
Horace ‡	4	168	580	38	211	50	285	380	32	275	50
Herman	4	125	423	20	270	42	220	421	20	322	42
Jack	4	163	398	28	353	45	223	364	30	345	45
Pete	4	189	600	13	484	28	217	425	12	425	12
Jett	4	138	732	41	732	41	237	435	22	431	41
Jill	4	115	571	28	571	28	205	495	20	495	20
Emma	4	185	299	30	299	30	212	346	30	346	30
Gertrude †	4	172	378	6	116	34	198	420	34	420	34
Kett	4	124	345	20	345	20	233	320	13	316	20
Stella	4	200	400	15	387	36		473	25	473	25

* Diets were changed (e.g. cholesterol diet No. 1 to 2 or 2 to 4) without any intervening rest period; so the same set of blood lipid data suffices for "final" for one diet and "initial" for the succeeding diet.
† These animals were sickly, with refusal of food on the indicated diets.
‡ These animals were given a low-fat cholesterol-free diet approximately one week prior to killing.

TABLE 5.—*An Abstract of Table 4, Emphasizing the Great Individual Differences in Relative Susceptibility to Diet-Induced Hypercholesteremia* *

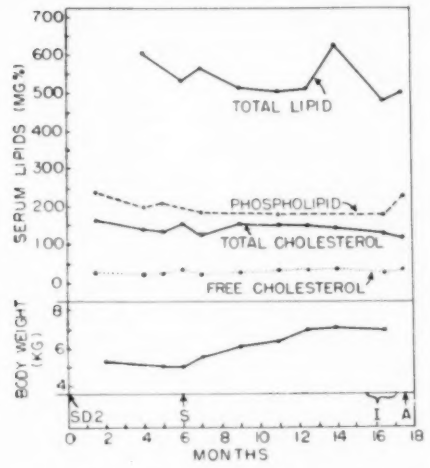
Animal	Control Serum Total Cholesterol		Total Period Given Cholesterol Diets, Weeks	Maximum Serum Total Cholesterol, Mg. %	Time Required to Elevate Mean Control Serum Total Cholesterol, Weeks†				Susceptibility to Hypercholesterolemia
	Determinations, No.	Mean, Mg. %			Twofold	Threefold	Fourfold	Fivefold	
Emma	4	150	30	239	...				Low
Olive	10	147	69	274	...				Low
Dorothy	1	164	28	397	9	...			Low
Henry	7	134	28	276	20	...			Low
Irma	1	129	95	248	44	...			Low
Josephine	4	140	97	394	4				Moderate
Gertrude	2	163	34	378	6				Moderate
Jack	2	172	45	398	12				Moderate
Stella	1	200	36	400	15				Moderate
Kett	2	125	20	345	4	20	...		Moderate
Herman	3	146	42	423	13	20	...		Moderate
Smiley	1	120	56	413	4	52	...		Moderate
Jill	5	159	28	571	4	20	...		High
Pete	4	155	28	600	5	13	13	...	High
Mary	1	154	58	586	4	16	18	...	High
Horace	2	138	52	580	16	35	38	...	High
Jett	3	131	41	732	5	5	12	20	High
Betty	10	125	105	669	3	21	26	34	High

* As an example of interpretation of this table, Mary attained a twofold elevation of her serum total cholesterol after 4 weeks of the cholesterol diet, a threefold elevation after a total of 16 weeks, and a fourfold elevation 2 weeks later (after a total of 18 weeks of the diet). † (...) indicates that values of this magnitude were never attained.

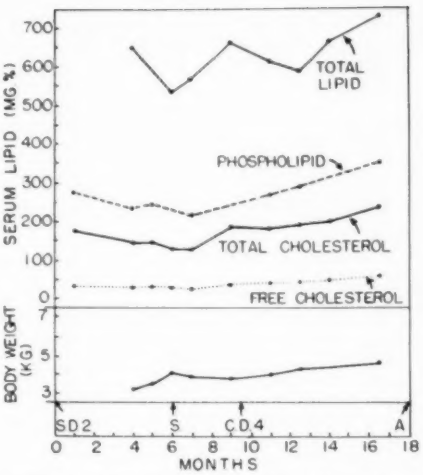
the terminal cholesterol levels are frequently less than the maximal because of terminal sickness with associated anorexia, or in some instances owing to the withdrawal of the high-fat, high-cholesterol diet several days before killing the animal, as part of a related study on hepatic cholesterol synthesis.

Table 5 is essentially an abstract of Table 4 to emphasize certain interesting features. One very interesting characteristic of the monkeys is the marked difference between individuals in their responses to the same diet. The animals are easily categorized into an arbitrary, but perhaps useful, classification of low, moderate, and high susceptibility to diet-induced hypercholesteremia. Some animals (Irma, Olive, Emma) showed very low susceptibility (or, conversely, marked resistance) to the hypercholesteremic effect of a high-fat, high-cholesterol diet. In general these less susceptible animals ate less than some of the highly susceptible animals; yet they ate enough to maintain their weight, and two showed a slight gain in weight. Some of our fattest and most robust animals with excellent appetites showed only moderate susceptibility (Stella, Herman), whereas others that also ate well, yet without any marked obesity, showed a high susceptibility (Betty, Pete, Jett). A serum total cholesterol of 300 mg.% was chosen as the arbitrary dividing line between low and moderate susceptibility; this is approximately twice the average control value for most of the animals. The line between moderate and high susceptibility was set at 500 mg.%, which is a little more than three times the average control level. Animals with levels in excess of this were classified in the high-susceptibility group. We do not intend to suggest by this arbitrary classification that nature has designed monkeys with three types of response to dietary fats; we merely hope to emphasize that monkeys do show definite and great differences in their responses to a high-fat, high-cholesterol diet. Animals undoubtedly show a continuous spectrum of responses, but for purposes of contrasting extremes and also for selection of animals for additional metabolic and balance studies, it seems useful to break the spectrum up into specific categories, which can be defined by a range of values and can be given an average or mean value and thus easily compared with one another.

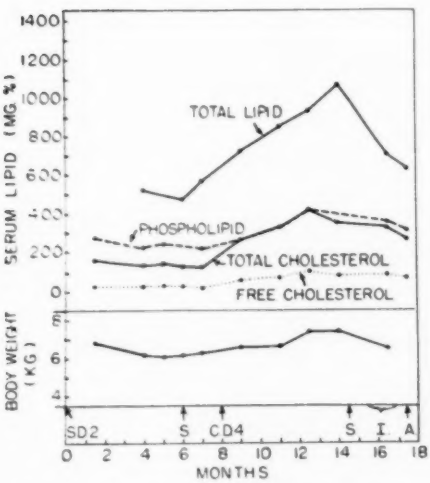
The susceptibility of an animal may show an apparent increase, with prolonged exposure to the high-fat diet, as in the case of Smiley, who for a long time showed low susceptibility but finally made the moderate group. However, this change in susceptibility occurred only after she developed an



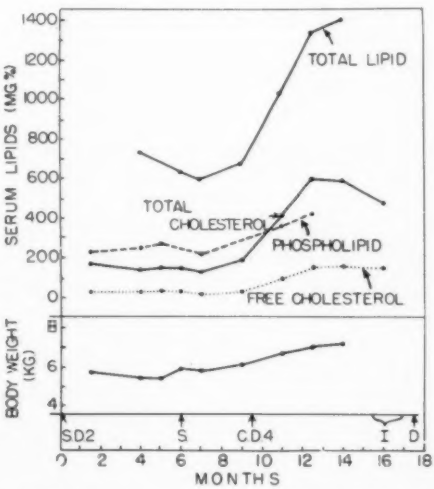
Graph 3.—Serum lipids in a monkey (Sylvester) maintained with a low-fat, low-cholesterol diet (Stock Diet No. 2). *S. D. 2* indicates Stock Diet No. 2; *S*, surgery; *I*, illness (tuberculosis); *A*, autopsy. Although the autopsy was necessitated by diffuse pulmonary tuberculosis, x-rays and skin tests were negative three months earlier.



Graph 4.—Response of a monkey (Emma) to a high-fat, high-cholesterol diet (Cholesterol Diet No. 4, which was moderately well eaten), showing a low susceptibility to hypercholesterolemia. *S. D. 2* indicates Stock Diet No. 2; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery; *A*, autopsy (because of contact with tuberculosis; no symptoms; x-rays negative).



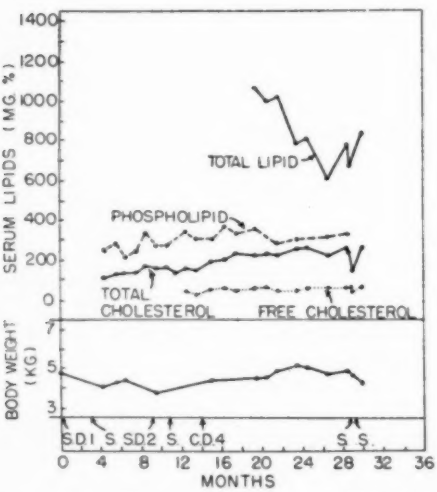
Graph 5.—Response of a monkey (Herman) to a high-fat, high-cholesterol diet (Cholesterol Diet No. 4, which was generally well eaten), showing a moderate susceptibility to hypercholesteremia. *S. D. 2* indicates Stock Diet No. 2; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery; *I*, illness (tuberculosis); *A*, autopsy (for tuberculosis).



Graph 6.—Response of a monkey (Pete) to a high-fat, high-cholesterol diet (Cholesterol Diet No. 4, which was well eaten), showing a high susceptibility to hypercholesteremia. *S. D. 2* indicates Stock Diet No. 2; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery; *I*, illness (tuberculosis); *D*, death (from tuberculosis).

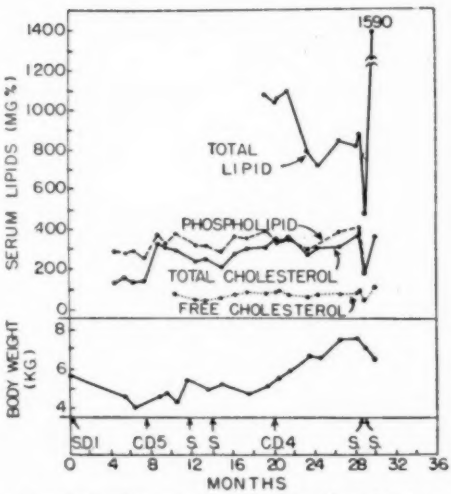
appetite for the diet and began to eat well enough to show some gain in weight. With Cholesterol Diet No. 4, animals generally ate well from the start; most of them very early gave an impression of being "susceptible" or "resistant," and prolonged feeding did not change the early impression based on the extent and rapidity of blood lipid changes.

Graphs 3, 4, 5, and 6 contrast the blood lipid data of four animals of the second group studied, illustrating the four groups of control and low, moderate, and high susceptibility to diet-induced hypercholesteremia. Tuberculosis prematurely interrupted the careers of these four animals, and given



Graph 7.—Response of a monkey (Olive) to prolonged cholesterol feeding, showing a low susceptibility to hypercholesteremia. *S. D. 1* indicates Stock Diet No. 1; *S. D. 2*, Stock Diet No. 2; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery.

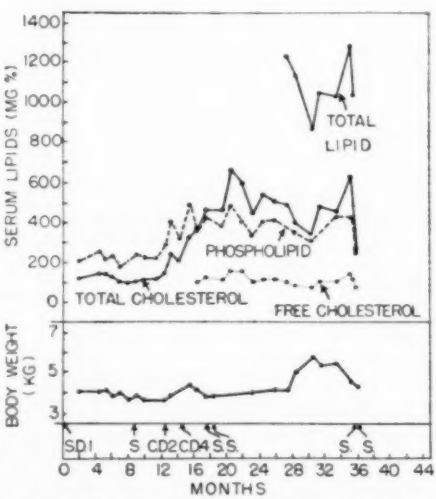
the diet for a longer time, Herman might have exceeded 500 mg.%. However, the difference between Pete and Emma was very striking from beginning to end and would probably have existed indefinitely. Graphs 7, 8, and 9 contrast three animals of the first group, which have been studied longer than any other. Two of these three are surviving and are still eating Cholesterol Diet No. 4 well. The graphs illustrate the



Graph 8.—Response of a monkey (Josephine) to prolonged cholesterol feeding, showing a moderate susceptibility to hypercholesteremia. *S. D. 1* indicates Stock Diet No. 1; *C. D. 5*, Cholesterol Diet No. 5; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery.

permanence of the difference in response shown by these animals.

Our study has not permitted a statistical analysis of any differences in the relative susceptibility (to diet-induced hypercholesteremia) of male versus female; however,



Graph 9.—Response of a monkey (Betty) to prolonged cholesterol feeding, showing a high susceptibility to hypercholesteremia. *S. D. 1* indicates Stock Diet No. 1; *C. D. 2*, Cholesterol Diet No. 2; *C. D. 4*, Cholesterol Diet No. 4; *S*, surgery.

TABLE 6.—*Serum Free Cholesterol Levels in Control and Cholesterol-Fed Monkeys* *

Group of Animals	Regimen	Animals, No.	Determinations, No.	Serum Free Cholesterol, Mg. % Range	Mean	Standard Deviation	F. C. T. C., Mean Ratio †
Control	Stock diet	16	71	21–50	33	3.8	0.21
Low susceptibility	Cholesterol diet	3	19	36–63	53	7.4	0.25
Moderate susceptibility	Cholesterol diet	6	35	43–104	84	11.3	0.25
High susceptibility	Cholesterol diet	5	37	68–189	107	21.9	0.23

* Terminally, Sheba and Gertrude (2 of the animals with fulminating pulmonary tuberculosis) showed free cholesterol-total cholesterol ratios of 0.44 and 0.60, respectively. These ratios were obtained primarily by a decrease in previously elevated total cholesterol levels, with little change in the absolute concentration of free cholesterol.
† Free cholesterol-total cholesterol.

we have noted no obvious differences. Both males and females have shown degrees of susceptibility varying from low to high. The "low susceptibility" group appears somewhat disproportionately female, but this may be an artifact due to a disproportionate loss of males from surgery and disease.

Graph 9 illustrates the marked fluctuation of serum total cholesterol values that occur occasionally, frequently associated with surgery or sickness but sometimes with no known cause. Fluctuations of 100 mg.% or more were fairly common in hypercholesteremic animals. No positive correlation with menstruation could be demonstrated. Probably some fluctuations were caused by unobserved periods of decreased ingestion of the diet.

Free Cholesterol.—The initial determinations of free cholesterol in 16 animals averaged 33.1 mg.%, with a free cholesterol-total cholesterol ratio of 0.20. These animals usually ate our control diet (Stock Diet No. 2) for four to six weeks prior to the initial blood sample. The average of all determinations of free cholesterol in control animals was 32.5 mg.%, with a free cholesterol-total cholesterol ratio of 0.21 (Table 6). Hypercholesteremic animals showed increases in free cholesterol that were generally proportional to the increase in total cholesterol, with a silght tendency to elevation of the free cholesterol-total cholesterol ratio in animals with moderate to high hypercholesteremia. Except for two animals dying from tuberculosis (Sheba and Gertrude, Table 6), the free cholesterol-total cholesterol ratios never showed any remarkable changes. The difficulty of interpreting small changes in free cholesterol-total cholesterol ratio is accentuated by its ready variation with several conditions, including red cell hemolysis and enzymatic activity (serum or bacterial) if the samples are stored unfrozen some time before the analysis is run.[62]

Lipid Phosphorus.—Lipid phosphorus determinations were performed throughout the study, and phospholipid concentration was calculated using the standard factor of 25, as suggested by the Nomenclature Committee of the American Society for the Study of Arteriosclerosis.[63] Control animals averaged 9.45 mg.% lipid phosphorus (Table 7); this value times 25 equals 236 mg.% phopholipid. The milligrams per cent of phospholipid always exceeded (by an average of 83.5 mg.%) the milligrams per cent of total cholesterol in control animals.

TABLE 7.—*Serum Lipid Phosphorus in Control and Cholesterol-Fed Monkeys*

Animals	Regimen	Animals, No.	Determinations, No.	Serum Lipid Phosphorus, Mg. % Range	Mean	Standard Deviation	M. S. P., Mg. % *	P.-T. C. Mean Ratio †	P.-F. C., Mean Ratio ‡
Controls	Stock diet	15	45	7.0–13.9	9.5	1.4	236	1.61	7.7
Low susceptibility	Cholesterol diet	4	26	8.9–16.8	12.3	1.2	308	1.49	5.9
Moderate susceptibility	Cholesterol diet	7	32	10.6–19.7	14.7	1.7	368	1.10	4.4
High susceptibility	Cholesterol diet	5	20	13.6–19.8	15.8	1.7	396	0.85	3.7

*Mean serum phospholipid (calculated as lecithin).
† Phospholipid-total cholesterol.
‡ Phospholipid-free cholesterol.

This is illustrated in Graph 3; the same relation also generally held for hypercholesteremic animals of the "low susceptibility" group (Graphs 4 and 7). In the "moderate susceptibility" animals, the cholesterol levels increased proportionately more than the phospholipid levels; as seen in Graphs 5 and 8, the two levels frequently coincided. The "high susceptibility" animals showed a proportionately still greater increase of cholesterol, with the milligrams per cent of total cholesterol exceeding the milligrams per cent of phospholipid (Graphs 6 and 9). Table 4 shows the peak phospholipid levels attained, which frequently did not occur at the same time as the peak cholesterol levels; this is indicated by the differences in "weeks on diet" (Table 4) required to reach the respective peak levels. None of our animals ever attained a phospholipid concentration in excess of 500 mg.%; four values approached 500 mg.%, but none went beyond that. However, higher levels probably would have accompanied higher cholesterol levels, as indicated by the recent report of a cholesterol-fed monkey that achieved levels of 800 to 900 mg.% phospholipid.[33] Table 7 presents the P:C ratios (milligrams per cent of serum phospholipid divided by milligrams per cent of serum total cholesterol), calculated largely from data shown in Graphs 2 through 9. The ratios of milligrams per cent of serum phospholipid divided by milligrams per cent of serum free cholesterol have also been tabulated, inasmuch as this relation has been reported to be more constant than the other ratio in cases of hypercholesteremia in humans.[64]

Total Lipid.—Fatty acid determinations early in the study indicated an average concentration of 0.94 mM. (approximately equivalent to 262 mg.%) of fatty acid per 100 ml. of whole blood in control young adult rhesus monkeys. This technique was given up because of technical difficulties together with our early despairing of producing significant hypercholesteremia. Procedures for the measurement of total lipid are in general notoriously inaccurate and nonspecific,[65] but for the detection of gross changes in total lipid (or neutral fat) the tedious labor of some of the more refined methods is a definite handicap. Our needs were adequately met by the introduction of Kunkel's simple and rapid technique for determination of total lipid. By subtracting from the milligrams per cent of total lipid the sum of the milligrams per cent of free cholesterol plus the milligrams per cent of cholesterol esters plus the milligrams per cent of phospholipid, one obtains a value which largely reflects the concentration of neutral fat in the serum; milligrams per cent of "neutral fat" = milligrams per cent of total lipid − ([milligrams per cent of total cholesterol × 1.67] + [milligrams per cent of lipid phosphorus × 25]). The factor 1.67 is used to estimate the milligrams per cent contribution of the fatty acid component of the esterified cholesterol. The quotation marks around "neutral fat" simply give recognition of the indirectness of measurement and the nonspecificity of this fraction. Control monkeys averaged 233 mg.% of serum "neutral fat" (Table 8), which appears to compare well with 262 mg.% of whole-blood fatty acids. Hypercholesteremic animals showed elevation of total lipid concentration, but this was always accomplished by elevation of cholesterol and phospholipid, with no elevation of "neutral fat" and no significant lactescence.

During part of the study, lipoprotein determinations were obtained and showed a severalfold increase of the −S 25-40 class (this corresponds to the S_f 12-20 class of Gofman and associates).[66] Details of these data will be published later.

Comment

This study demonstrates that rhesus monkeys are susceptible to moderate and severe hypercholesterolemia induced by ingestion of a diet rich in cholesterol and total fat.

TABLE 8.—*Serum "Neutral Fat" in Control and Cholesterol-Fed Monkeys* *

Group of Animals	Regimen	Animals, No.	Determinations, No.	Serum Neutral Fat (Calculated from Total Lipid), Mg. %		
				Range	Mean	Standard Deviation
Controls	Stock diet	14	25	102–339	233	42
Low susceptibility	Cholesterol diet	2	8	66–487	227	166
Moderate susceptibility	Cholesterol diet	6	19	21–381	187	53
High susceptibility	Cholesterol diet	4	11	133–384	236	76

* Quotation marks around "neutral fat" indicates our awareness of the nonspecificity of this fraction. See text under total lipids.

However, negative results are easily obtained in such studies, simply by failure to present the fat in a form that the animals will eat readily. A one-food, one-meal technique appears most effective for inducing hypercholesteremia. By presenting the basic diet (Stock Diet No. 2), cholesterol and fat in the form of a single homogeneous preparation, there is no way for the animals to avoid ingestion of the fat. In addition, even when a suitable diet is used, occasional individual animals show a very low susceptibility (high resistance) to the hypercholesteremic effects of the diet. Thus, if any small group of test animals chanced to include several resistant ones, the average hypercholesteremic response would be low and erroneous conclusions could thereby be drawn concerning the response of monkeys to that regimen. These two factors probably account for the generally negative results of earlier studies by other workers.

The hypercholesteremic response of the monkeys in this study appears to be due solely to the excess of dietary lipids, inasmuch as all known essential dietary factors were present in concentrations adequate to support good health indefinitely in control animals. Ascorbic acid and folic acid were added to the diet to insure against deficiencies reported to result from commercial processing of food. Vitamin K was added empirically after it was noticed on several occasions that some animals bled freely from their gums during their struggles against capture and restraint for venipuncture. Pyridoxine was added to rule out the possibility of pyridoxine deficiency as a factor in any hypercholesteremic and/or atherosclerotic responses that might be observed.

In this preliminary work, little attention was devoted to the role of saturated or unsaturated fats. The first problem at hand appeared to us to be the development of a dietary regimen by which monkeys can be induced to eat fat-rich foods. This has been accomplished, and we feel that the basic diet reported herein is ideally suited to studying various types of fat as to their respective roles in lipid metabolism and hypercholesteremia.

Sex plays no known role in the susceptibility of rhesus monkeys to diet-induced hypercholesteremia. This paper describes moderate to severe hypercholesteremia produced in both male and female animals. The data do not permit a statistical evaluation of the relative susceptibility of male versus female.

Age may be a significant factor. Clinically, hypercholesteremia and atherosclerosis generally are minimal in the first two decades of life and become progressively more important through the next three or four decades. Whether this indicates a progressive reduction in the capacity of the individual for handling fats or simply reflects the gradual accumulation of fat from a diet that always more or less exceeded individual metabolic capacities is not known. Young, prepubertal rhesus monkeys are known to be susceptible to hypercholesteremia,[59] but our study was restricted to sexually mature animals. Animals with body weight over 3.5 kg. (female) or 4.5 kg. (male) were almost always sexually mature, with estimated ages of 3½ to 6 years, corresponding approximately to a human age of 12 to 20 years. Three females with an estimated equivalent "human" age of at least 30 to 40 years showed no greater susceptibility to

hypercholesteremia in their short period of study than most of the younger animals. Thus, this study provides no experimental implication of age as an important factor in the development of diet-induced hypercholesteremia.

Given Cholesterol Diet No. 4, most monkeys showed a fairly rapid elevation of serum cholesterol to a plateau which appeared more or less stable and characteristic for each individual. With increasing intake of exogenous cholesterol, the monkey probably absorbs a decreasing percentage of the total, thus providing an upper limit for the total amount of cholesterol taken into the system. In a single female monkey (Jill) a daily ingestion of 10 gm. of cholesterol a day for two months produced an elevation of serum cholesterol no greater than that previously produced by ingestion of 2 to 3 gm. of cholesterol per day. A similar observation was reported by Mann and Andrus.[33] This phenomenon of an absorption plateau has been demonstrated very clearly in rats.[67]

In contrast to Greenberg and Rinehart,[59] our monkeys have shown permanent elevation of the serum cholesterol levels for as long as they continued adequate ingestion of the cholesterol diet. As indicated by our experiences, a probable explanation of the decrease in serum cholesterol observed in their monkeys after the 55th week of the experimental diets is a period of sickness and/or voluntary fasting.

The fluctuation of serum lipid levels, with depression following sickness, surgery, or decreased food intake from any other cause, is reasonable and is in accord with clinical findings.[68] The depression of serum cholesterol after the onset of tuberculosis in some of the monkeys is also in accord with clinical data.[37] The decreased food intake and the weight loss seem to us to be completely adequate to explain the observed depression of blood lipids.

The elevation of free cholesterol in the cholesterol-fed monkeys is nearly parallel with the elevation of total or ester cholesterol. The free cholesterol-total cholesterol ratio has been indicated to be of value in estimating hepatic function, disturbance in the ratio supposedly being associated specifically with hepatic disease.[69] This study confirms that dietary fat, in the presence of normal liver function, does not appreciably alter the free cholesterol-total cholesterol ratio. The percentage of free cholesterol is slightly lower in monkeys (free cholesterol-total cholesterol ratio, about 0.2 to 0.25) than in humans studied by the same methods in our laboratory (free cholesterol-total cholesterol ratio, about 0.25 to 0.35). The significance of or reason for this species difference is unknown.

Several studies have suggested that downward deviations of the phospholipid-cholesterol ratio may be of considerable importance in the genesis of atherosclerosis. Jackson and Wilkinson demonstrated that in clinical hypercholesterolemia due to divergent conditions the phospholipid-free cholesterol ratio is more nearly linear than the phospholipid-total cholesterol ratio.[64] In the monkeys reported herein the serum phospholipid-cholesterol ratio decreases as the cholesterol level increases. The decrease in phospholipid-free cholesterol ratio, in the more hypercholesteremic animals, slightly exceeds the decrease in the phospholipid-total cholesterol ratio. The difference, however, is minimal because of the relative constancy of the free cholesterol-total cholesterol ratio. Thus, in hypercholesteremic monkeys, in the absence of hepatic or metabolic disease, the phospholipid-total cholesterol ratio is as constant as the phospholipid-free cholesterol ratio.

The elevation of the serum cholesterol without a concomitant elevation of serum neutral fat is noteworthy. Work by Keys and Anderson[70] emphasizes the total fat content of the diet rather than the cholesterol content as the most important etiologic factor in clinical hypercholesteremia and atherosclerosis. There has as yet been no clear explanation of how excessive dietary

fat, other than cholesterol, produces hypercholesteremia. A possible explanation may be increased absorption of cholesterol (biliary, intestinal, and dietary) following the ingestion of extradietary neutral fat or fatty acids. If the serum neutral fat rose in response to the dietary fat and serum cholesterol rose secondarily in response to the elevated serum neutral fat, this might be a reasonable explanation. But this is certainly not the sequence of events in this study. The elevation of serum cholesterol appears to be the primary event in response to a diet high in total fat and in cholesterol. The effect of a diet high in total fat but very low in cholesterol is currently being studied in monkeys. The work to date is too preliminary to be discussed.

In some ways, to some degree, the response of monkeys to dietary fat and cholesterol simulates that of humans. Thus, striking individual variation is also seen in people; e. g., one person eats cholesterol- and fat-containing foods with impunity, while another appears to eat possibly less fat but yet suffers more and earlier complications of severe atherosclerosis. The explanation for their different responses may reside in genetics, or stress, or exercise. Such individual variations in humans probably have an explanation similar to the individual variations in monkeys. Stress and exercise appear comparable in our monkeys and thus appear to be ruled out as significant factors. And even if the explanation is "different genetics," there must still be specific quantitative or qualitative biochemical differences between the individuals. It seems reasonable and useful to think in terms of quantitative differences in the total capacity of the individual for disposing of cholesterol. This total capacity would be the sum of the individual capacities of several different biological processes and metabolic pathways. Individual pathways include absorption, endogenous synthesis of cholesterol (and its facility for suppression by dietary cholesterol), conversion of cholesterol to bile acids, excretion of cholesterol and bile acids in the feces, and synthesis of sex and adrenal hormones and possibly other substances from cholesterol. Efforts to quantitate these individual capacities in monkeys are in progress.

The magnitude of the problem of human vascular disease may justify a little hopeful speculation at this point. Certain species of animals, and individuals within species, have a high resistance to dietary cholesterol and fat. These animals may differ from "susceptible" animals in some but not all biochemical pathways. The differences are probably quantitative rather than qualitative. By quantitating the major known metabolic pathways, in both resistant and susceptible animals, it might be possible to demonstrate a causal relation between the degree of susceptibility and the quantitative capacity of some single "critical" pathway. If this pathway possessed a similar role in the human and if it were susceptible to measurement, a useful clinical test would be possible. The application of a single test to an individual to quantitate the capacity of this critical pathway might permit prediction of his potential resistance or susceptibility to the hypercholesteremic effects of dietary cholesterol. Such a test applied to relatively young people could give a useful early warning if their metabolic capacity were such that they would need to restrict their dietary fat intake to prevent the eventual development of hypercholesteremia and atherosclerosis. Rhesus monkeys show considerable individual variation, and we think they are ideal for this type of investigation, as a preliminary to similar clinical investigations.

Summary

This paper presents some blood lipid data obtained from 30 adult rhesus monkeys which were subjected to various fat-containing diets for periods of several months to three years.

Several types of high-fat, high-cholesterol diets were tried. It was found to be difficult, but essential, to present the fat diet in

a form palatable to monkeys. An efficient, effective diet was developed, with use of a commercial ground monkey food (Stock Diet No. 2) as the basic ration, to which were added supplements of butter and cholesterol.

A one-food- one-meal-per-day technique was found highly useful. The experimental fat-containing diet was prepared to contain all nutritional requirements, and almost no other food was given to the experimental animals. This helped to insure ingestion of the fat and prevented the animals from subsisting solely on fruit rations while giving project members the delusion that the fat diet was being well eaten.

With use of the diet and the feeding technique described above, the developement of significant hypercholesteremia in most monkeys became not only possible but relatively easy. Serum total cholesterol levels of 300 to 600 mg.% developed in one to three months in the majority of animals that were adequately tested.

The hypercholesteremic responses varied, some animals showing a "low" susceptibility to diet-induced hypercholesteremia and a moderate number showing either "moderate" or "high" susceptibility. It is felt that these variations in response to dietary fat are comparable to the variations clinically observable in human subjects.

The hypercholesteremia is interpreted as due chiefly to the excess of dietary cholesterol, rather than to the excess of dietary neutral fat. We favor this interpretation because (*a*) the serum neutral fat was usually normal or slightly subnormal, even in animals with marked hypercholesteremia, and (*b*) in preliminary studies, dietary fat alone, without cholesterol, has produced no significant hypercholesteremia (see Addendum).

Grossly and microscopically visible atherosclerosis developed in most animals subjected to moderate (300 to 500 mg.%) or high (over 500 mg.%) hypercholesteremia for a period of several months or longer. In the same or similar animals, intimal lipid deposition was accelerated by subjecting segments of the vessel wall to transient freezing injury. These and additional observations will be reported in more detail in a future publication.

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Addendum

Current studies have afforded some observations, which, although preliminary, are interesting and pertinent to the next to last paragraph in the summary. Ten adult rhesus monkeys receiving 42% of their calories as fat were divided into two groups, with one group receiving butter as the source of fat and the other group, margarine (vegetable fats). Butter provided each animal with approximately 50 mg. of cholesterol per day. No additional cholesterol was given. The animals given margarine received essentially no dietary cholesterol. After 8 to 12 weeks of these diets, the serum cholesterol of the animals ingesting butter showed percentage increases (over their individual average control value) of 22%, 2%, 42%, 99%, and 29% (average of 39%). The serum cholesterol of the animals ingesting margarine showed percentage increases of 15%, 15%, 18%, 6% and 4% (average of 13%). These data, all that we have to date, support the postulated direct relationship of dietary cholesterol to serum cholesterol, an excess of the former inducing an excess of the latter, with only a secondary role assigned to dietary neutral fat or fatty acids.

Also, several additional studies on monkeys have been recently published. Portman and colleagues carried out an interesting series of dietary studies[71] and demonstrated markedly greater hypercholesteremic effects of lard as compared to corn oil, when fed to Cebus monkeys along with α-protein and cholesterol (0.1 gm. of cholesterol per 100 Cal. of diet). This, along with numerous other recent experimental studies, emphasizes the possibility that our initial failure to induce marked hypercho-

lesteremia in rhesus monkeys may have been due in part to our use of corn oil as the source of dietary fat. A later publication, by Portman and Sinisterra,[72] discussed some radioisotopic studies on certain aspects of cholesterol metabolism in Cebus monkeys. Among other things, their observations included measurement of the biological half-life of serum cholesterol. In monkeys fed a corn oil-no cholesterol diet, the intravenous injections of cholesterol-4-C^{14} gave die-away curves which showed a biological half-life of serum cholesterol of 8.8 days. However, monkeys which received cholesterol-4-C^{14} by intragastric intubation showed die-away curves with two distinct components, an initial rapid phase (half-life 1.9 to 6.6 days), followed by a slower secondary phase (half-life 17 to 34 days). These half-life values are similar to those reported for humans, with London and Rittenberg [73] and Gould [74] supporting the eight-day value, and Hellman et al.[75] supporting the 20+-day value. Thus monkeys resemble humans even to the extent of showing comparable inconsistencies when the same pathway or phenomenon is measured by different techniques.

Kritchevsky and McCandless [76] reported a very great degree of spontaneous fluctuation in the serum total cholesterol levels of monkeys ingesting a basal diet. The maximum fluctuation in their animals exceeded the maximum that we have observed, but our experience, as well as theirs, emphasizes the need for using control values that represent the average of several blood samples over a period of several months, instead of taking a single control sample and then starting the animal on an experimental regimen. This precaution is all the more necessary when one tries to compare regimens that may produce only slightly to moderately different serum cholesterol levels.

Several reports from the Randolph Air Force Base in Texas have supplied excellent ideas on the prevention and control of tuberculosis in rhesus monkeys.[77] Their current technique of mixing isoniazid in the diet so that each animal ingests approximately 20 mg. of isoniazid per kilogram of body weight per day, to minimize or prevent outbreaks of tuberculosis, appears to be quite sound and well worth adopting if one's experimental studies will permit the continued use of this drug.

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Cystic Lesions of the Adrenal Glands

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Cysts of the adrenal gland are rare lesions which are generally detected at necropsy. In reviewing the American literature, Siceluff [1] found 19 cases in which the diagnosis of adrenal cyst was made preoperatively.

The first reported case was that of Greiselius, of Vienna, in 1670 (quoted by Doran [2]). In 1951 Wahl found 9 cysts in a series of 13,996 autopsies.[3] In 1955 Adderley, Grimes, and Whitehead found 77 reported cases and added 4 of their own.[4]

Several systems of classification have been proposed.[5-8] That of Terrier and Lecène includes (1) parasitic cysts, (2) true glandular cysts, (3) cystic adenomas, (4) serous or lymphatic cysts, and (5) pseudocysts. Parasitic cysts are rare. Barnett studied 1617 cases of Echinococcus cysts and found adrenal involvement in less than 0.5%.[9] Dew mentions in his book on hydatid disease that he found only a single instance of adrenal involvement.[10] True glandular cysts occur even more rarely in man, only a single case having been reported, that by Sick,[11] in 1903. Cystic degeneration of adenomas of the adrenal cortex probably accounts for many of the smaller cysts found at autopsy. Serous and lymphatic cysts are more frequent and account for many of the larger cysts detected clinically. Pseudocysts are produced as a result of hemorrhage into the gland and may well be the commonest.

We wish to propose a slight revision in the classification based on practical rather than theoretical considerations.

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Basically all cysts may be classified as either true or false cysts. True cysts include the rare glandular cysts and the commoner cysts formed by dilated lymphatics or vascular channels that are lined by endothelium. False cysts, or pseudocysts, include cystic degeneration of adenomas or malignant tumors, hemorrhage or necrosis of the cortex, and parasitic cysts.

A. True cysts
   1. Glandular cysts
   2. Lymphangioma or lymphangiectasis
   3. Hemangioma

B. Pseudocysts resulting from
   1. Hemorrhage into cortex
   2. Necrosis and cystic degeneration of benign or malignant primary tumors
   3. Parasitic infection

During the past 25 years only 2 authentic cysts of the adrenal cortex have been encountered in 11,000 autopsies performed at Wayne County General Hospital. During the latter part of the same period two cases were encountered in the surgical material of this and another hospital.

Report of Cases

CASE 1.—A 41-year-old white woman was found on routine examination to have a soft-tissue mass in the left flank. Pyelograms made by intravenous and retrograde injections indicated the presence of a left suprarenal mass which depressed the left kidney (Fig. 1). At operation the mass was found not to be attached to the kidney. The cyst ruptured during dissection, allowing approximately 350 ml. of thick opaque fluid to well into the operative site. The postoperative course was uneventful.

The specimen consisted of a thin-walled collapsed sac that had measured about 10 cm. in diameter. The wall was composed of thin translucent gray-white tissue containing bright yellow nodules resembling adrenal cortex, measuring up to 2 mm. in greatest dimension. The inner surface was smooth and glistening.

Microscopically the wall was composed of connective tissue (Fig. 2) and an oc-

Fig. 1 (Case 1).—"Intravenous" pyelogram showing downward displacement of left kidney by large suprarenal mass.

casional nest of adrenal cortical cells. The wall was devoid of elastic fibers. Within the fibrous tissue layer were many mononuclear cells containing golden brown granular pigment in the cytoplasm. The diagnosis was pseudocyst of adrenal gland, secondary to old hemorrhage.

Case 2.—A 29-year-old white woman was suddenly seized with a severe pain in the left side of the flank and abdomen while watching a parade. She was admitted to DePaul Hospital, Cheyenne, Wyo. Physical examination disclosed a large, tender, soft, and slightly movable mass in the left upper portion of the abdomen, measuring approximately 20 cm. in diameter. Pyelograms made by intravenous and retrograde injections revealed the presence of a soft-tissue mass in the upper part of the abdomen which displaced the left kidney to the right. At operation a fluctuant thin-walled cyst was found in the left suprarenal area, adherent to but easily separated from the adjacent kidney. In the course of the dissection the cyst ruptured, allowing the escape of much cloudy odorless thin fluid containing many flakes of gray friable tissue. The cyst and suprarenal gland were removed from their attachment by a pedicle near the upper pole of the kidney.

The specimen consisted of a collapsed sac which could be reconstructed to measure 20 by 17 by 15 cm. The wall was irregular in thickness but did not exceed 1 mm. Stretched tightly over one area of the cyst was a distorted adrenal gland which measured 8 by 5 by 0.5 cm. The gland on section was composed of two layers of cortex, each less than 1 mm. wide, separated by medulla 1 to 3 mm. in thickness.

Microscopically the wall of the cyst was composed of collagenous connective tissue with several nodules of adrenal cortical tissue on the outer aspect. The inner surface had no epithelial lining, but here and there were particles of eosinophilic necrotic debris. Suitably stained sections failed to reveal the presence of iron, while the wall was composed of connective tissue but contained no elastic fibers. Focal lymphocytic infiltration was observed around small vascular channels in the periadrenal connective

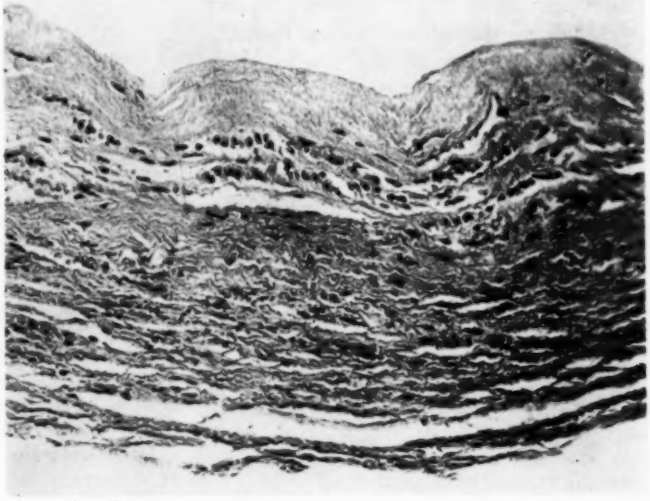

Fig. 2 (Case 1).—Section of wall of cyst showing fibrosis and chronic inflammatory reaction but no cortical cells or endothelial lining. Reduced 15% from mag. ×250.

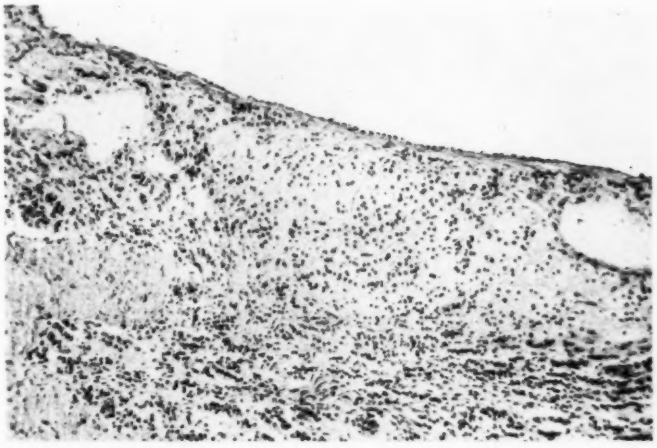

Fig. 3 (Case 3).—Cyst wall lined by endothelium, lying adjacent to adrenal cortex. Reduced 30% from mag. ×140.

tissue. The diagnosis was unilocular pseudocyst of the adrenal gland.

CASE 3.—A small cyst of the adrenal gland was an incidental finding at autopsy in a 61-year-old white man who died suddenly. He had severe coronary arteriosclerosis without demonstrable coronary occlusion or myocardial infarction. The left adrenal gland was slightly enlarged and at its anteroinferior margin contained a cyst, measuring 2.5 cm. in diameter, which was filled with thin clear fluid and possessed a smooth lining.

Microscopically the cyst wall was composed of several layers of adrenal cortical cells at the periphery and a thin layer of fibrous tissue lined by flattened endothelium (Fig. 3). Portions of intact adrenal cortex and medulla were found adjacent to the cyst. Stains were negative for iron and elastic tissue. The diagnosis was lymphangiectatic cyst of the adrenal glands.

CASE 4.—A multilocular adrenal cyst (Fig. 4) was an incidental finding at autopsy in a 95-year-old white woman who died of bronchopneumonia. The right adrenal gland was considerably enlarged, weighing 42 gm. and measuring 8 by 6.5 by 2.5 cm. On section the cortex was orange and varied from 2 to 3 mm. in thickness. A large cortical cyst, measuring 2.6 cm. in greatest dimension, was surrounded by and communicated with a number of smaller satellite cysts that measured up to 0.8 cm. in diameter. The cysts were filled with thin clear fluid. The lining was smooth and glistening except for excrescences of gray-yellow soft friable tissue attached to the wall. The walls of the cysts contained many calcified plaques. Chemical analysis of the cyst fluid revealed total protein, 5.1 gm. per 100 ml. (albumin, 3.5 gm.; globulin, 1.6 gm.); total cholesterol, 82 mg. per 100 ml. (cholesterol esters 54 mg., or 66% of the total); sodium, 139 mEq. per liter; potassium, 23 mEq. per liter; and chloride, 100 mEq. per liter.

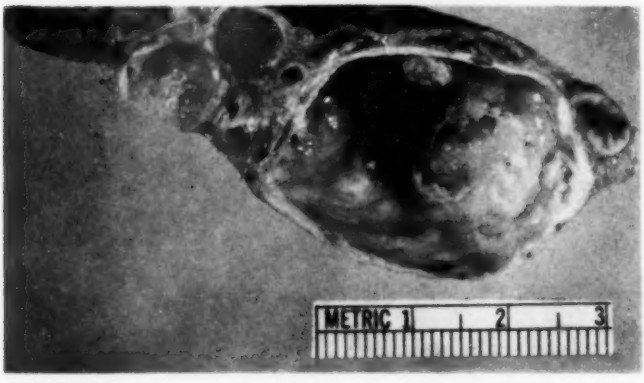

Fig. 4 (Case 4).—Cross section of a multilocular cyst. Note necrotic cortical tissue adherent to inner wall of largest cyst. The adjacent adrenal cortex is increased in thickness.

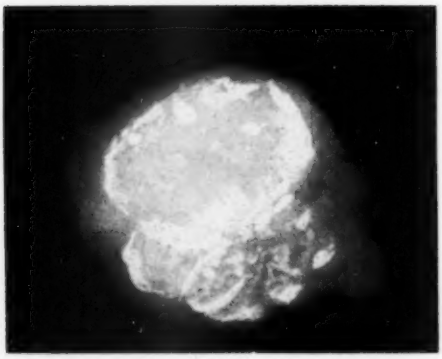

Fig. 5 (Case 4).—Radiogram of gland showing extensive calcification in walls of cysts.

An x-ray film of the removed gland revealed extensive calcification in the walls of the cysts (Fig. 5).

Microscopically the wall of the cyst was composed of a thick layer of hyalinized connective tissue with many areas of calcification. The cyst had no endothelial lining. The adrenal cortex peripheral to the cyst was mildly hyperplastic. The excrescences on the inner surface of the wall were composed of eosinophilic, acellular, amorphous debris (Fig. 6). Several small nodules of hyperplastic cortical cells were noted in the cortex. Special stains failed to reveal the presence of iron or elastic fibers in the cyst wall. The diagnosis was cystic degeneration of adrenal cortex.

Comment

Cysts of the adrenal gland have been reported in persons of all ages but seem to be commoner in older people. They are usually found at autopsy. In this hospital most of the autopsies are performed upon persons in the older age groups. Cysts have been encountered on the right side as often as on the left.[12] As a rule they do not give rise to symptoms, except as the result of pressure. Adrenal insufficiency associated with bilateral cystic lesions of the adrenal

Fig. 6 (Case 4).—The inner surface of the cyst is covered by a mass of necrotic debris. The inner zone of the cortex has been partially replaced by fibrous tissue; × 250.

gland has been reported in children.[13] Brindley and Chisolm have reported two instances of cystic tumors of the adrenal gland associated with Cushing's syndrome, the signs and symptoms of which regressed after adrenalectomy.[14]

Cysts vary considerably in size, ranging from a few millimeters to 30 cm. in diameter. The cyst described by Gardiner and associates contained 11 liters of fluid.[15] False cysts that arise either from central hemorrhage or necrosis of normal or abnormal glandular tissue are the commonest, and these attain the largest size. Cysts produced as a result of dilatation or proliferation of the vascular or lymphatic channels usually are small and encountered only at autopsy. The absence of an epithelial lining excludes the diagnosis of true angiomatous cyst.

Many of the pseudocysts have focal calcification that has followed necrosis of lipid-containing adrenal cortex. Drucker and Rodriguez reported bilateral adrenal calcification which was discovered on routine roentgen examination of the chest.[16] Their patient, a child, was observed for two years and at autopsy was found to have bilateral adrenal cysts with calcification, presumably the result of hemorrhage or infarction. Hemorrhage or infarction in otherwise normal adrenal glands could account for the changes observed in our Cases 1 and 2, although there is nothing to suggest the cause of such hemorrhage. In Case 1 the pigment within macrophages may have come from old hemorrhage. In Case 2 blood pigment could not be demonstrated in the cyst wall, but the eosinophilic material adherent to the lining probably represents coagulated protein. In 1906 Terrier and Lecène proposed the concept that hemorrhagic pseudocysts were late sequelae of infection; in their time, hemorrhage in the adrenal cortex was fairly common in infectious diseases. It is of interest that more cases of this type were reported at the end of the last and the early part of the present century, when infections were not as well controlled as now. There was no history of past infectious disease of sufficient severity to warrant speculation that this was the cause of adrenal hemorrhage in either Case 1 or 2. Adderley and associates[4] postulate that hemorrhage may result from a nonspecific angiitis and/or from a congenital weakness of the vessel wall. In one of their cases the periadrenal connective tissue contained focal infiltration of lymphocytes, plasma cells, and eosinophils. For this reason the hemorrhage was thought to be secondary to angiitis. In our Case 2 focal infiltrations of lymphocytes, predominantly perivascular, were present in the outer portion of the cyst wall as well as in the adjacent fibroadipose tissue. We regard this inflammation as a reaction to the contents of the cyst rather than a vasculitis, since there was no other anatomic evidence of vascular disease. After the original hemorrhage, a hematoma may imbibe fluid and become very large before giving rise to symptoms. The presence of many thin-walled vascular channels in the cyst wall, such as were seen in Cases 1 and 2, would facilitate this process.

Case 3 represents a cystic lymphangiectasis of the adrenal gland which possibly arose from lymphatics in the medulla, since lymph channels in the human adrenal gland are found only in the capsule and medulla. According to Rabson and Zimmerman,[17] most of these cysts are actually hamartomas because the absence of proliferating endothelium excludes a diagnosis of true neoplasm.

The multiocular cyst found in Case 4 was located within a hyperplastic adrenal gland containing focal nodular hyperplasia. The focal calcification in the cyst wall suggests previous necrosis of lipid-containing tissue. The thick walls of the cyst probably prevented the imbibition of fluid by the contents of the cyst. Cystic degeneration of a cortical adenoma is rare, but, if an adenoma should become large enough, it is conceivable that it could become cystic. On

the contrary, cystic degeneration of medullary tumors is fairly common.

Summary

Cysts of the adrenal gland are rare. Four additional cases are presented, one representing a true cyst and three, false cysts. Few adrenal cysts give rise to symptoms. False cysts are usually larger and may or may not give rise to symptoms, depending on their size. Cysts rarely, if ever, have endocrine activity.

A slight revision in the classification of the adrenal cysts is suggested, based on practical rather than theoretical considerations.

Dr. S. A. Giovale and Dr. Francis A. Barrett Jr. gave permission for use of clinical records from the files of DePaul Hospital, Cheyenne, Wyo., and Mr. Adrian Brothers and Miss June Morris made the photographs.

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Glycogen Infiltration ("Hydropic Degeneration") in the Pancreas

A Review

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The problem of the pathogenesis and significance of "hydropic degeneration" of pancreatic B-cells was thought, until recently, to have been solved. This lesion has been reported to occur in almost every type of diabetes, both human and experimental. It has been generally accepted as being a degenerative lesion, signifying exhaustion of B-cells due to excessive insulinogenesis which if prolonged would ultimately result in their destruction.[1-14] In 1922, Allen [4] had stated that this was the only example of disintegration of a cell system associated with increased functional activity. However, he only with difficulty reconciled this interpretation with the fact that hydropic degeneration also occurs in pancreatic duct epithelium. In subsequent years the possible significance of this lesion in duct epithelium has been glossed over. More recently it has been found that the vacuolation interpreted as "hydropic degeneration" is due to artifactual removal of glycogen deposited within duct epithelium and B-cells.[15] This makes the original "exhaustion" hypothesis less plausible, since, to our knowledge, in no other instance does glycogen infiltration reflect a degenerative process. These inconsistencies prompted a review of this problem and further experimental work which led to a reevaluation of this phenomenon.

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The Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, and the Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

Hydropic Degeneration in the Human Pancreas

In 1901, Weichselbaum and Stangl [16] described for the first time a peculiar vacuolation of the pancreatic islets of comatose diabetic patients, which they [17] subsequently interpreted as being a degenerative lesion due to liquefaction of the B-cell granules and leading eventually to atrophy of these cells. Since none of the then available staining techniques enabled the demonstration of glycogen, mucin, or other subtances in the vacuoles, the term "hydropic degeneration" was coined to indicate the presumably aqueous character of the contents. Subsequently, Weichselbaum [18] reported vacuolation of the islet cells in 98 of 183 diabetic patients (53%) as one of the most frequent lesions of the pancreas in diabetes. Several later authors [19-22] confirmed these observations, but in a comparatively smaller percentage of cases (8% to 40%). Others [23,24] were unable to find this lesion in the diabetic pancreas, while in one instance [25] vacuolation was observed in the islets of both diabetic and nondiabetic subjects. Warren [26] reported pancreatic islet vacuolation in only 22 out of 484 cases of diabetes (4.5%) and stated that this change occurred as frequently before as after the discovery of insulin. He furthermore suggested that postmortem autolysis may be confused with this lesion. Gomori [27] failed to observe vacuolation in a single diabetic pancreas but found such changes in five nondiabetic patients, one of whom had received large amounts of intravenous dextrose solution. Very recently, on the other hand, Hartroft,[28] using phase-contrast

microscopy, reported B-cell hydropic change in 8 of 46 diabetic patients (18%).

"Hydropic Degeneration" in Experimental Diabetes

In 1913, Allen [1] studied the morphologic alterations in the pancreas of dogs rendered diabetic by 90% partial pancreatectomy in an attempt to determine whether changes occur which could account for the development of diabetes in these animals. He observed in the islet cells deficiency of cytoplasm and frequently pyknotic or naked nuclei, but no change was noted in the acinar tissue. In general, the visible lesions corresponded to the duration and severity of the diabetes and were hypothesized to result from overstimulation and exhaustion, owing to the fact that 10% of the pancreas was required to perform the work of the entire organ. Alternative explanations for the morphologic changes were considered to be the hyperglycemia itself, or possibly some effect of ketosis, inanition, or some other unknown alteration in the body metabolism associated with the development of diabetes. However, subcutaneous dextrose which in some instances produced prolonged glycosuria did not evoke any morphologic alteration in the pancreas. This was thought to make it unlikely that the pancreatic islet lesion was due to a simple excess of circulating sugar. It was also shown that in normal dogs neither starvation nor inanition produced similar morphologic changes. These findings seemed to provide the evidence that had been sought as to the relationship between the pancreatic islets and the development of diabetes and led to the view that the islet changes are the cause of the diabetes rather than the reverse.

At about the same time Homans [2,3] studied the development of experimental diabetes in the partially pancreatectomized cat and dog, also in an attempt to determine the relationship of the islets to diabetes. Utilizing specific stains, he demonstrated loss of secretory granules, swelling, and an apparent hyperactivity of B-cells in diabetes. Furthermore, he reported that in the more severely diabetic animals there was hydropic degeneration of B-cells, which he related to that previously described in the human. No alterations were observed in other islet cells or in ductular epithelium.

It was considered that the hydropic cells contained fluid and that the B-cell changes were progressive, starting with degranulation, followed by hydropic degeneration, eventually leading to their destruction and to permanent diabetes. This sequence was taken as confirmation of the "exhaustion" theory and provided evidence that the B-cell is responsible for the production of insulin. Homans also demonstrated that a low-carbohydrate diet prevented the morphologic changes apparently further supporting this theory.

In 1922, Allen [4] reported further on the problem of hydropic degeneration, including its evolution in 90% partially pancreatectomized dogs. He showed that the rate of development of the anatomic changes varied with the clinical status of the animals. In severe diabetes a period of four to seven days was required for the first appearance of vacuolation. In a month maximum vacuolation was seen, and in six weeks to two months all B-cells disappeared from the pancreas. During these studies it also was found that there was vacuolation of duct epithelium during the diabetic state. Furthermore, proliferation of small pancreatic ducts and cell cords was observed, which was interpreted as an atempt at repair. Since the small ducts do not have an internal secretory function, and since they did not suffer any further degeneration, he considered their vacuolation as due to exhaustion of a possible proliferative rather than of an endocrine function. In a following study, Allen,[5] by intravenous dextrose administration, attempted unsuccessfully to produce pancreatic-cell vacuolation in nondiabetic dogs and to increase the vacuolation in diabetic animals. He also reported that a high carbohydrate intake, although it causes hyperglycemia and

glycosuria, will not cause hydropic degeneration if this would not have occurred with a low-carbohydrate intake. Furthermore, in his hands phlorhizin administration did not prevent the degeneration of the islets of diabetic animals even though the blood sugar was kept at or continuously below normal levels. From these experiments it was concluded that "hydropic degeneration" results from a humoral stimulus which cannot be the increased glycemic level and therefore is probably a deficiency of circulating insulin. In 1923, Copp and Barclay [29] demonstrated that insulin administration reverses the vacuolation in the pancreas of diabetic partially pancreatectomized dogs apparently by relieving the "functional strain" on the B-cells.

In subsequent years various authors [30-34] observed that injections of crude anterior pituitary extracts produced diabetes in experimental animals. Young [35] showed conclusively that if the diabetes which occurs during the injection of anterior pituitary extracts is sufficiently severe and if it is allowed to persist it will continue indefinitely even if the injections cease. Richardson and Young [6] reported degranulation and hydropic degeneration of B-cells and in some instances complete disappearance of the B-cells in anterior-pituitary-treated dogs. Richardson,[7] in a more extensive study, found that there was some degree of vacuolation of intralobular duct epithelium in all anterior pituitary-treated dogs even in the absence of obvious diabetes or of anatomic changes in the B-cells. The significance of this finding could not be explained, but he felt that the term hydropic degeneration was not applicable to duct epithelial cells, since they are not destroyed in pituitary diabetes, as are the B-cells. Ham and Haist [8] reported a time-sequence study of the morphologic changes in the pancreas after the administration of crude anterior pituitary extract. They observed first degranulation, then hydropic change, and then eventual disappearance of the B-cells. This sequence of events was used as evidence to support the "exhaustion" theory of the pathogenesis of hydropic degeneration. These workers also noted vacuolation of duct epithelium but did not attribute special significance to it. Others [36] reported in the rabbit slight vacuolation of the intralobular ducts but none in B-cells after treatment with anterior pituitary extract. Lukens and his co-workers [9,10] demonstrated that "hydropic degeneration" and eventual disappearance of islets will occur in partially pancreatectomized cats after prolonged administration of anterior pituitary extracts. Duct epithelial changes were not discussed. It was also shown that diet, phlorhizin, and insulin administration prevent these islet lesions and the development of permanent diabetes. These findings were taken to indicate that the "exhaustion" of islet cells in anterior pituitary diabetes is due to hyperglycemia rather than to hypoinsulinemia. This viewpoint seemed to be supported by the fact that dextrose administration causes hydropic degeneration in the pancreas of guinea pigs [37] and cats and also permanent diabetes in cats.[11,38] Purified growth hormone has also been demonstrated to cause degranulation, vacuolation, and atrophy of B-cells, with the development of permanent diabetes.[39-41]

In alloxan diabetes which is due to B-cell destruction vacuolation occurs in the epithelium of the intralobular pancreatic ducts in dogs.[42] In alloxanized rabbits some workers [43,44] described hydropic degeneration in occasional islet cells but did not mention the ducts, whereas in another study [45] no alteration was found in the duct epithelium. On the other hand, Duff, et al.[46] showed that after prolonged alloxan diabetes in rabbits there was vacuolation of residual B-cells and of intralobular ductular epithelium. In general the lesion occurred simultaneously at both sites; however, in one instance ductular vacuolation was seen even though no B-cells were present. It was also noted in this study that hydropic degeneration of B-cells and ductular epithelium persisted in association with more or

less severe diabetes for periods of up to one year without histologic evidence of any disintegration of the affected cells. In view of this, it was doubted that the hydropic cells within the islets were actually altered B-cells. It was felt that they possibly originated by proliferation from undifferentiated ductular epithelium which subsequently became vacuolated. These authors, furthermore, demonstrated that the vacuolation was reversible by adequate treatment of the diabetic state with insulin. In other work [47] it was shown that vacuolated islet cells in alloxan diabetes actually may be altered B-cells which still contain intact mitochrondria, macular zones, and Golgi apparatus.

In adrenocortical-steroid-induced temporary diabetes in rabbits [48-50] and guinea pigs [51-53] hydropic degeneration of pancreatic B-cells and ductular epithelium has also been observed. Houssay [14] reported vacuolation of B-cells and the production of permanent diabetes in 80% to 85% partially pancreatectomized dogs after administration of cortisone and hydrocortisone. More recently [54] it has been shown that cats treated with fludrocortisone developed temporary diabetes with hydropic change in islets. Vacuolation of B-cells and permanent diabetes has also been induced in partially pancreatectomized dogs by thyroid feeding.[12,13] These latter studies do not, however, mention any morphologic alterations in pancreatic duct epithelium.

Pancreatic Glycogen Infiltration in Diabetes

During a study of sites of glycogen deposition in human diabetes, Warren,[55] in 1938, described its presence in pancreatic duct epithelium in 73% of non-insulin-treated diabetics and in 45% of insulin-treated diabetics with complicating infection. On the other hand, no duct epithelial glycogen was found in nondiabetics or in insulin-treated diabetics. Richardson,[7] in 1940, reported that in dogs made diabetic by anterior pituitary extract some duct vacuolation was due to glycogen deposition, some to fat, and only some to hydropic change. In 1951, Toreson [15] demonstrated for the first time, and it was soon confirmed,[56] that "hydropic degeneration" of pancreatic B-cells and duct epithelium consists actually of vacuolation due to artifactually extracted glycogen deposits.

More recently,[49,50] the development of vacuolation and glycogen infiltration in the pancreas of cortisone-treated rabbits was studied. Glycogen infiltration was not found in the normal rabbit pancreas. As diabetes evolved, vacuolation and glycogen deposition appeared first in the duct epithelium and then in B-cells, but only after severer and prolonged hyperglycemia (Figs. 1 to 5). In other studies [57] in hydrocortisone and hydrocortisone and growth hormone treated rabbits a similar sequence was observed. Studies in dogs [40,58] showed that in the untreated animal there were sometimes glycogen-containing vacuoles in the duct epithelium (Figs. 6 and 7). Furthermore,[40] the daily administration of purified growth hormone * to the intact dog caused increasing vacuolation and glycogen infiltration of ductular epithelium proportional to the duration of treatment. It was only after a longer period of diabetes that small amounts of glycogen appeared within the B-cells. A similar progression [59] was found in dogs made diabetic by partial pancreatectomy alone or partial pancreatectomy followed by growth hormone or cortisone administration (Figs. 8 to 11).

In an unpublished study, by us, diabetes has been produced in rabbits by daily administration of crystalline glucagon † conjointly with small doses of cortisone. In these animals the degree of vacuolation and glycogen infiltration of ducts and B-cells corresponded also with the duration and severity of the diabetic state. In mild dia-

* Purified growth hormone was a gift from the Endocrine Study Section, National Institute of Health.

† Crystalline glucagon (Lot No. 258-234B-54-2) was supplied by the Lilly Research Laboratory, Eli Lilly and Company, Indianapolis, through Dr. W. R. Kirtley.

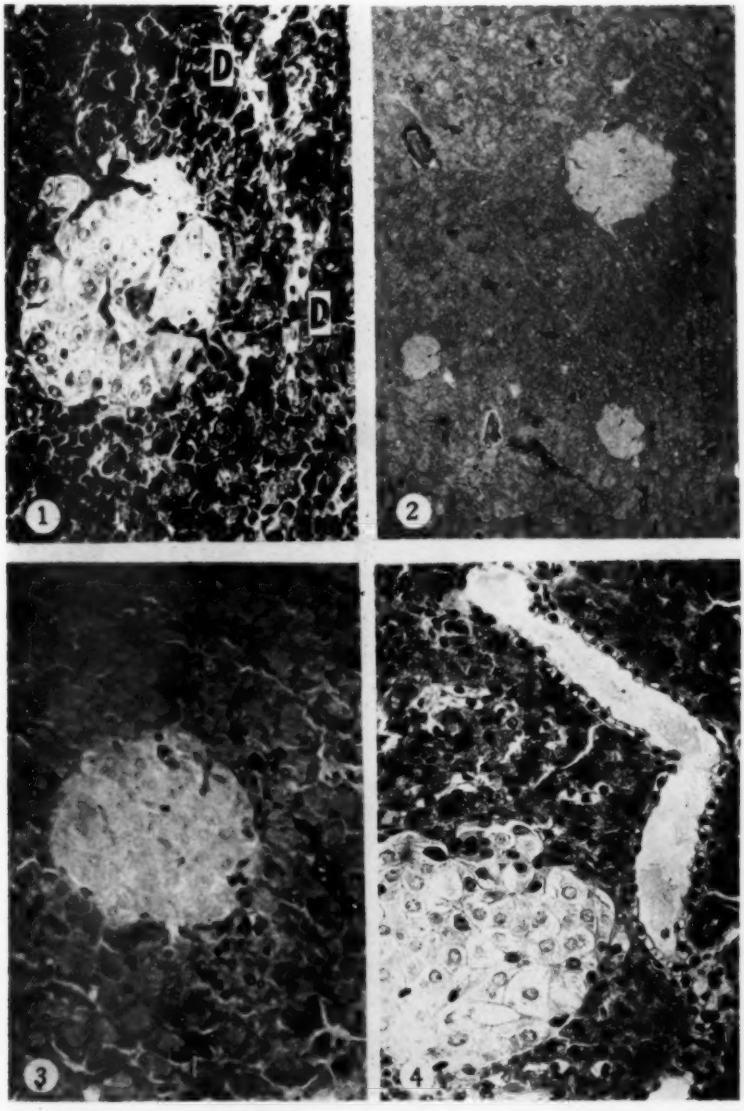

Fig. 1.—Pancreas of diabetic rabbit treated with cortisone acetate, 5 mg. per kilogram, intramuscularly, for 10 days. There is vacuolation of ductular epithelium (*D*) and degranulation but no vacuolation of B-cells. Modified Masson-trichrome stain; reduced about 15% from mag. × 375.

Fig. 2.—Low power of same pancreas as in Figure 1, showing glycogen deposits (black) in ductular epithelium but not in B-cells. Periodic acid-Schiff-trichrome; reduced about 15% from mag. × 90.

Fig. 3.—Higher power of same pancreas as in Figure 2, showing glycogen deposits (black) in ductules. Periodic acid-Schiff-trichrome stain; reduced about 15% from mag. × 375.

Fig. 4.—Pancreas of diabetic rabbit treated with cortisone acetate, 5 mg. per kilogram, intramuscularly, for 21 days, showing vacuolation in both ductular epithelium and B-cells. Modified Masson-trichrome stain; reduced about 15% from mag. × 375.

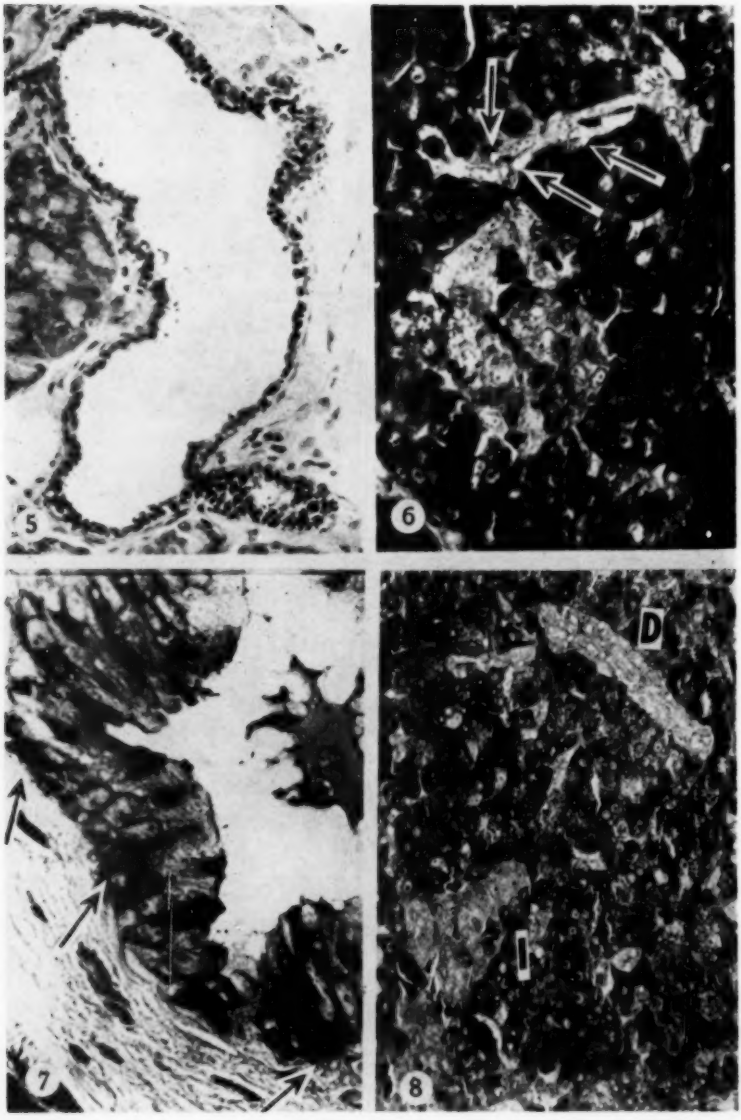

Fig. 5.—Pancreas of same rabbit as in Figure 4, showing glycogen (black) in epithelium of larger duct. Periodic acid-Schiff-trichrome stain; reduced about 15% from mag. × 375.

Fig. 6.—Pancreas of normal dog, showing paranuclear vacuolation of ductular epithelium (arrows). Modified Masson-trichrome stain; reduced about 15% from mag. × 525.

Fig. 7.—Pancreas of normal dog. Glycogen in epithelium of large duct (arrows). Periodic acid-Schiff-trichrome stain; reduced about 15% from mag. × 1040.

Fig. 8.—Pancreas of dog treated with 3 mg. per kilogram of purified growth hormone daily for six days. There is vacuolation of ductular epithelium (*D*) but not of the islet (*I*). Modified Masson-trichrome stain; reduced about 15% from mag. × 375.

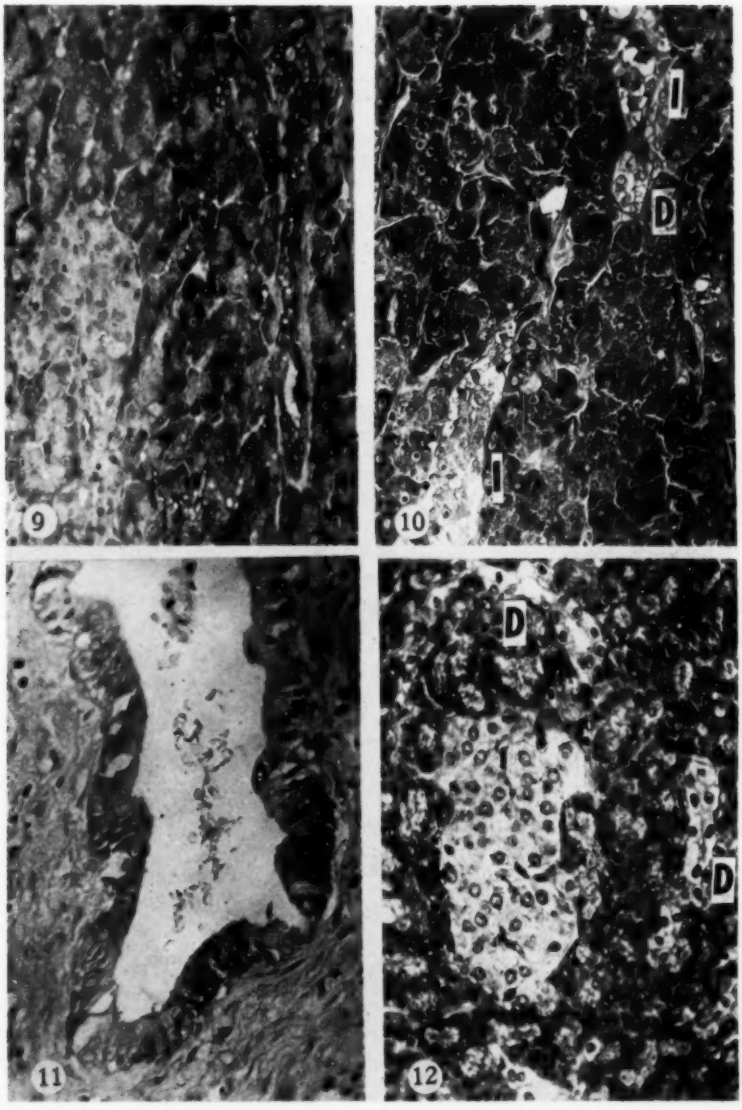

Fig. 9.—Pancreas of same dog as in Figure 8, showing glycogen (black) in ductular epithelium but not in islet. Periodic acid-Schiff-trichrome stain; reduced about 15% from mag. × 375.

Fig. 10.—Residual pancreas from partially depancreatized dog who after the eighth postoperative day received 8 mg. per kilogram of cortisone acetate for 38 days. There is extensive vacuolation of both B-cells (*I*) and duct epithelium (*D*). Aldehyde-fuchsin-trichrome stain; reduced about 15% from mag. × 375.

Fig. 11.—Pancreas from same dog as in Figure 10, showing vacuolation of larger duct. Aldehyde-fuchsin-trichrome stain; reduced about 15% from mag. × 375.

Fig. 12.—Pancreas of rabbit treated simultaneously daily with 3 mg. of crystalline glucagon and 1 mg. per kilogram of cortisone acetate, showing marked ductular (*D*) but only slight islet vacuolation. Modified Masson-trichrome stain; reduced about 15% from mag. × 375.

betes only ductular epithelium and an occasional B-cell were affected (Fig. 12). In severer diabetes the extent of the B-cell alterations was greater.

Glycogen infiltration of B-cells has also been reported after prolonged intraperitoneal administration of dextrose in cats.[60] However, glycogen infiltration of duct epithelium was not discussed. In these experiments the degree of glycogen storage in the B-cells paralleled the duration of the injections and particularly the amounts of dextrose administered.

Comment

The original hypothesis of Allen [1,4,5] that "hydropic degeneration" in the pancreas is a regressive morphologic response to excessive functional activity was accepted and supported by most workers.[6-14,56,61-63] This theory seemed to be borne out by the observation that metadiabetes is produced by administration of crude anterior pituitary extracts,[6-8,35,61] adrenocortical steroids,[14] thyroid extract,[12,13] and prolonged dextrose administration.[11] In each instance the morphologic evolution which serves as the basis for the development of diabetes was thought to be first degranulation, then vacuolation, and eventual disappearance of the B-cells.

The early workers [2-5,29,64] supported the view that hypoinsulinemia was the stimulus for increasing insulin output from B-cells. At the present time, it is still believed that B-cell degranulation in the diabetic state reflects increased insulin output, with increased B-cell functional activity. However, the stimulus is thought to be hyperglycemia, rather than hypoinsulinemia.[41,65-74] A considerable body of evidence also suggests that hyperglycemia per se is the pathogenetic factor responsible for "hydropic degeneration" of B-cells.[9-11,37,38,69]

The fact that both degranulation and vacuolation of B-cells have a pathogenetic mechanism in common and that one lesion usually follows the other does not necessarily imply that one evolves from the other. This is particularly emphasized by the accumulated evidence that vacuolation in the pancreas is neither exclusively nor primarily a B-cell lesion.[4,5,7,8,40,48-50,57,59] Furthermore, some residual granulation of B-cells has been seen simultaneously with vacuolation in a partially pancreatectomized growth-hormone-treated dog.[41] It has also been shown that the hypoglycemic sulfonylureas which apparently stimulate increased pancreatic insulin output caused degranulation but not vacuolation of the B-cells.[41,70-75]

The finding that "hydropic degeneration" consists actually of the deposition of glycogen in B-cells and duct epithelium would further imply that B-cell degranulation and vacuolation are separate entities. Toreson [15] questioned the exhaustion theory of hydropic degeneration and suggested that this lesion might represent merely one component of the widespread pathologic deposition of glycogen which occurred in diabetes. An alternative explanation, tentatively proposed, was that in the diabetic pancreas glycogen infiltration may be an indication of deranged regeneration of islets. This idea was put forward, since glycogen was found also in ductular epithelium, which serves as the principal histogenetic source of differentiated islet cells in fetal and probably also in extrauterine life. Another explanation was that its pathogenesis was similar to that of glycogen nephrosis in that possibly the duct epithelium and B-cells absorb dextrose from the surrounding fluid and that an increase of dextrose concentration of these fluids would result in deposition of glycogen within the cells. In a further study, Duff and Toreson [60] apparently showed that exogenous insulin prevents the development and causes the regression of glycogen infiltration of B-cells and intralobular ductules despite the presence of an elevated blood sugar level. They concluded that this supports the view that hypoinsulinism provides the stimulus for the B-cell hyperfunction, which would ultimately lead to hydropic degeneration. This formulation of their conclusion is rather contradictory in

view of the lack of functional activity of intralobular ductules. Therefore, unless the assumption is made that the mechanism of glycogen infiltration in B-cells differs from that of the ductular system, a hypothesis which relates insulinogenic activity and hydropic change seems to be completely unwarranted. Furthermore, it has been shown [69] that direct introduction of dextrose into the pancreaticoduodenal artery causes "hydropic change" in islets despite the increased circulating insulin which also occurs.

The finding that glycogen infiltration and vacuolation occurred in the duct epithelium of cortisone-diabetic rabbits prior to its appearance in the B-cells also seemed to be completely at variance with the idea that this change represented a regressive morphologic response of B-cells to prolonged excessive functional activity.[49,59] It seemed to support strongly the idea that this form of deposition of glycogen is not a degenerative change but is merely one component of the more widespread pathologic deposition of glycogen which occurs in diabetes. The observation that the prior deposition of glycogen in duct epithelium is apparently a universal phenomenon for most forms of diabetes further validates this viewpoint.[40,57,59] Also the fact that in the dog glycogen normally may be present in duct epithelium [40,58] seemed to indicate that its increased deposition in this structure in diabetes is merely an accentuation of a normal process due to increased amounts of available glucose.

According to this viewpoint the pancreatic glycogen deposits in diabetes would be pathogenetically related to those observed in renal tubular epithelium and in muscle. The vacuolation of renal tubular epithelium in diabetes was considered a degenerative process until Ehrlich [81] demonstrated that the vacuoles actually contained glycogen. Furthermore, it is noteworthy that originally Allen [1] compared the morphologic appearance of "hydropically" degenerated pancreatic cells with the appearance of the cells of the renal tubules in glycogen nephrosis. At the present time renal tubular glycogen deposits are considered to be a reversible metabolic phenomenon which is an expression of a high urinary glucose concentration.[82] Furthermore, the increased cardiac glycogen observed in both human and experimental diabetes is accepted as a mere reflection of the elevated glycemic level.[83]

The exact reasons for the specific sites of deposition of glycogen in the diabetic pancreas are not quite clear. It has been suggested [40,50,59] that the earlier deposition of glycogen in duct epithelium might be a result of increased concentration of dextrose within the external pancreatic secretion in diabetes [84] and absorption of dextrose from the lumen by duct epithelium. The fact that glycogen appears more readily in B-cells than in acinar tissue might be due to the markedly greater vascularity of the islet tissue.[59] This is supported by the observation that the glycogen seems to appear first at the vascular pole of the B-cells.[57] The question is still left unanswered, however, as to why glycogen is not found in A-cells or D-cells. Furthermore, proliferating ductular epithelium usually does not contain any glycogen.[49] This would lead to the conclusion that B-cells and resting ductular epithelium have some common metabolic denominator which allows for the accumulation of glycogen within these cells.

This change in viewpoint that B-cell vacuolation rather than being a degenerative lesion due to exhaustion of insulinogenesis is merely an expression of glycogen infiltration as a consequence of hyperglycemia may have further implications. In other situations glycogen may accumulate within cells without causing their destruction. This is exemplified in von Gierke's disease and diabetes itself in the renal tubular epithelium. Allen [4] remarked on the lack of destructive effect of vacuolation on duct epithelial cells in the partially pancreatectomized dog. It has also been

shown in adrenal-steroid-treated guinea pigs[51,53] and alloxan-diabetic,[46] adrenal-steroid-treated,[48-50] adrenal-steroid-and-growth-hormone-treated,[57] and cortisone-and-glucagon-treated rabbits[85] that there may be marked hydropic change in the pancreas without associated B-cell destruction or the development of permanent diabetes. It therefore seems that the concept which suggests that glycogen infiltration in the B-cells is the pathogenetic mechanism which leads to their destruction and to permanent experimental diabetes may have to be revised.

Summary

The problem of "hydropic degeneration" in the pancreas is reviewed. With present knowledge it should be recognized that this term is a misnomer and that this lesion should rather be called glycogen infiltration. It has been found that in the normal dog as well as in human and experimental diabetes glycogen infiltration may frequently only be present in ductular epithelium. On the other hand, B-cell glycogen infiltration occurs as a comparatively late phenomenon which depends on the duration and severity of the diabetic state.

Taken in conjunction these observations are at variance with the heretofore generally accepted view that "hydropic degeneration" is an expression of functional exhaustion of B-cells. In the light of this the linking of B-cell degranulation and vacuolation as sequential events in the evolution of B-cell degeneration in diabetes can no longer be justified. These two lesions should rather be considered as independent manifestations of hyperglycemia, the B-cell degranulation indicating increased insulinogenesis and the glycogen infiltration being merely one component of the generally increased glycogen deposition in diabetes. Furthermore, since glycogen at other sites does not cause cell destruction, it is questioned whether infiltration of this material in B-cells is responsible for the development of the various forms of metadiabetes.

Mr. Herbert A. Fischler prepared the photomicrographs.

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Biological Studies of Dihydrocholesterol

III. Regression of Dihydrocholesterol-Induced Cholecystitis and Cholelithiasis in the Rabbit

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Introduction

Previous studies showed that cholecystitis and cholelithiasis can be produced in rabbits by incorporating 0.25% to 1% dihydrocholesterol in their regular diet.[1,2] It was further found that these lesions of the biliary tract could be prevented entirely or in part by the simultaneous administration of dehydrocholic acid.[3]

In the present study changes in the severity of the lesions of the biliary tract were measured after the causative agent (dihydrocholesterol) had been removed from the diet. This information was needed for evaluating the effect of therapeutic measures, such as drugs or dietary factors, on the development of the disease. The therapeutic effect of dehydrocholic acid was studied as part of the present investigation.

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Methods

Forty-two male rabbits weighing 1.5 to 3 kg were fed 1% dihydrocholesterol incorporated into a basal diet consisting of Purina Rabbit Chow Pellets to which 12% olive oil U. S. P. had been added. The animals were kept in individual cages and had access to food and water at all times. At the end of four weeks the animals were divided into three groups, C, S, and D; Group C consisted of 10 animals which were killed at the end four weeks' dihydrocholesterol feeding in order to determine the condition of their biliary tracts and to measure the concentration of tissue sterols *before* any regression had taken place. The remaining 32 rabbits were divided into two groups of 16 animals. One group (S) was fed the basal diet only; the other group (D) received basal diet plus 0.25% dehydrocholic acid. Rabbits from both of these groups were killed at intervals of 1, 2, 4, 6, 14, and 19 weeks, and the severity of the lesions of the biliary tract was determined. The extent of the regression of the lesions was estimated by comparison with the animals of Group C (which had received 1% dihydrocholesterol for four weeks) and by comparison with two additional groups of animals which had received no dihydrocholesterol. Group NS consisted of seven animals which had been fed the basal diet for four weeks, and group ND consisted of three animals maintained with

TABLE 1.—*Tissue Sterol Concentrations of Rabbits Maintained on Diets Not Containing Dihydrocholesterol*

Group	Diet	Duration of Regimen, Wk.	Animals, No.	Serum Sterol Concentration: Initial Cholesterol, Mg. %	Serum Sterol Concentration: Final Total Sterol, Mg. %	Serum Sterol Concentration: Final DHC in Total Sterol, % *	Liver Sterol Concentration: Total Sterol, Mg/Gm. †	Liver Sterol Concentration: DHC in Total Sterol, % *	Muscle Sterol Concentration: Total Sterol, Mg/Gm. †	Muscle Sterol Concentration: DHC in Total Sterol, % *
NS	Basal	4	7	42	51	8	3.31	16	0.62	13
ND	Basal + 0.25% dehydrocholic acid	6	3	92	76	3	2.42	7	0.66	23

* DHC indicates dihydrocholesterol.
† Mg/gm. wet weight.

TABLE 2.—*Effect of Four Weeks' Dihydrocholesterol Administration*

Group	Animals, No.	Initial Serum Cholesterol Concentration, Mg. %	Disposition at End of 4 Wk.	Dry Weight of Gallstones, Mg.	Serum Sterol Concentration: Total Sterol, Mg. %	Serum Sterol Concentration: Cholesterol, Mg. %	Serum Sterol Concentration: DHC in Total Sterol, % *	Liver Sterol Concentration: Total Sterol, Mg/Gm. †	Liver Sterol Concentration: DHC in Total Sterol, % *	Muscle Sterol Concentration: Total Sterol, Mg/Gm. †	Muscle Sterol Concentration: DHC in Total Sterol, % *
C	10	42	Killed	190	104	42	60	5.12	40	0.62	33
S	16	44	Given basal diet	..	128	58	55	--	--	--	--
D	16	45	Given basal diet + 0.25% dehydrocholic acid	..	110	50	55	--	--	--	--

* DHC indicates dihydrocholesterol.
† Mg/gm. wet weight.

the basal diet plus 0.25% dehydrocholic acid for six weeks (Table 1, result section).

The histologic and biochemical methods have been described in previous publications.[2,3]

Estimation of Pathologic Involvement.—In an attempt to evaluate the pathologic findings, the severity of the gross and microscopic lesions of the gallbladder and of the intra- and extrahepatic bile ducts was graded on an arbitrary scale. Ratings of 0 (no lesion) to ++++ (very severe lesion) were assigned to the following findings:

1. Cellular reaction and fibrous thickening of
   (*a*) gallbladder
   (*b*) extrahepatic bile ducts
   (*c*) intrahepatic bile ducts
2. Presence of concrements in
   (*a*) gallbladder
   (*b*) extrahepatic bile ducts
   (*c*) intrahepatic bile ducts
   (*d*) common duct

Table 4 lists these data in detail for each animal.

Results

Table 1 summarizes the tissue sterol concentrations of the two groups of animals (NS, ND) which had received diets containing no dihydrocholesterol. When these animals were killed they exhibited neither cholecystitis nor cholelithiasis. These data provide information on the normal tissue sterol levels of rabbits maintained with the high-fat basal diet, for comparison with the three groups of animals (C, S, D) which received "lithogenic" regimen.

Table 2 summarizes the serum sterol data obtained in the animals of Groups C, S, and D at the end of four weeks of dihydrocholesterol administration. The Table also lists the average dry weight of the gallstones and the concentrations of liver and muscle sterols of the 10 animals in Group C, which were killed at that time. All of the animals of this group (and presumably all of the 32 animals in Groups S and D) had cholelithiasis and cholecystitis as expected on the basis of previous studies, in which 100% of the animals so treated developed these lesions.

The administration of a diet containing 1% dihydrocholesterol had little effect on serum cholesterol concentration (Table 2).

TABLE 3.—*Weight of Gallstones and Concentrations of Tissue Sterols of Rabbits Given Regression Regimens*

Regression Diet	Regression Period, Wk.	Animals, No.	Serum Sterols: Total, Mg. %	Serum Sterols: DHC, % *	Liver Sterols: Total, Mg/gm.†	Liver Sterols: DHC, % *	Muscle Sterols: Total, Mg/gm.†	Muscle Sterols: DHC, %	Dry Weight of Gallstones, Mg.
Basal	1	2	96	61	8.48	44	0.56	40	95
	2	3	228	45	11.49	40	0.71	31	207
	4	3	60	40	2.44	20	0.49	20	113
	6	2	85	25	2.99	14	0.67	12	250
	14	2	80	12	3.56	13	0.64	8	0
	19	4	34	11	3.33	14	0.62	13	50 ‡
Basal + 0.25% dehydrocholic acid	1	2	116	67	6.14	37	0.69	38	120
	2	3	123	35	4.03	24	0.73	24	187
	4	3	64	4	2.47	7	0.67	16	73
	6	2	39	22	2.91	18	0.64	16	155
	14	2	60	18	6.30	16	0.64	12	45 ‡
	19	4	54	9	6.10	14	0.62	13	3 ‡

* DHC indicates dihydrocholesterol.
† Mg/gm. wet weight.
‡ Only one animal in this group had biliary calculi.

However, the dihydrocholesterol concentration of the serum rose so that at the end of four weeks 55% to 60% of the serum sterols consisted of dihydrocholesterol. The average liver sterol concentration likewise increased to about 5 mg. per gram of wet weight, again largely due to an accumulation of dihydrocholesterol. Muscle total sterol concentrations remained unchanged, but the proportion of dihydrocholesterol in muscle sterols rose to 33%. These data are similar to results obtained previously in three-week feeding experiments with 0.5% and 1.0% dihydrocholesterol.[3]

Table 3 summarizes the average serum, liver, and muscle sterol concentrations and the dry weight of the gallstones of rabbits killed 1, 2, 4, 6, 14, or 19 weeks after cessation of dihydrocholesterol feeding. Comparison with the data of Table 2 shows that the dihydrocholesterol content of the serum did not fall immediately when dihydrocholesterol feeding was discontinued: There was a further increase during Week 1, particularly in the animals receiving dehydrocholic acid. It is doubtful that this rise can be attributed to mobilization of dihydrocholesterol prior to excretion because muscle and liver sterol concentrations exhibited a similar peak at Week 1. The data indicate that tissue sterol levels had returned approximately to normal (Table 1) at the end of 14 to 19 weeks except for the liver sterol concentrations of the animals receiving stock diet plus 0.25% dehydrocholic acid during the regression period. While the concentration of dihydrocholesterol and cholesterol in the livers of these animals of Group D at first fell rapidly toward normal values, the total sterol concentration rose again to values of about 6 mg. per gram toward the end of the experiment. This increase was due largely to an increased storage of cholesterol, not dihydrocholesterol. This effect may be ascribed to an increased absorption of endogenous cholesterol in rabbits receiving dehydrocholic acid. We have previously reported that this triketocholanic acid, like the naturally occurring bile acids, can promote tissue storage of cholesterol and dihydrocholesterol in the rabbit.[3] The high fat content of the diet may also have been involved in producing this result: Oil red O stains of the liver indicated that the increased liver cholesterol content was accompanied by an increase of sudanophilic material in this organ.

During the first six weeks of the regression experiment the average dry weight of the gallstones showed no definite trend either upward or downward. However, at the end of 14 weeks only one of four animals had gallstones, and at the end of 19 weeks two of eight animals retained their biliary calculi (Table 4). It is thus demonstrated that in the rabbit most experimentally induced gallstones can be removed from the gallbladder within a relatively short period.

Gross Pathology.—There was no difference between the gross appearance of the gallbladders of animals killed at the end of four weeks of dihydrocholesterol feeding and those killed during the first six weeks of the regression period. Some of the gallbladders were distended with thick cloudy bile; others were contracted about large concrements. In all instances the gallbladder wall was thickened and the mucosal pattern was irregular, owing either to exaggeration or to flattening of the folds. Often the serosal surface had a bead-like appearance, resulting from the presence of small concrements within Aschoff-Rokitansky sinuses. Hydrops was encountered in one animal (Rabbit 42, Table 4) in which the cystic duct was obstructed by calculi. Four animals (Rabbits 41, 45, 85, and 186) developed exaggerated mucosal folds partially encasing the firm concrement.

At the end of 14 and 19 weeks of regression, the thickening of the walls of the majority of the gallbladders had largely disappeared. However, residual signs of previous injury persisted in the form of minute outpouchings visible on the serosal

TABLE 4.—*Extent and Intensity of Cholelithiasis and Cholecystitis During Regression Experiment*

Rabbit No.	Regimen: Diet	Period, Wk.	Calculi Present in: Intrahepatic Bile Ducts	Extrahepatic Bile Ducts	Common Bile Duct	Fundus of Gallbladder	Cellular Reaction & Fibrous Thickening of: Intrahepatic Bile Ducts	Extrahepatic Bile Ducts	Gallbladder
37	1% dihydrocholesterol	4	+	0	0	++++	++++	0	++++
38			+	0	0	+++	++	+	+
39			0	0	++	+++	++	+	++
83			++	++++	++++	+++	+++	++	++
84			++++	++	0	+	++++	++++	++
85			0	0	0	++	+	++	++++
86			0	0	0	+	+++	++	++
185			++	++	+	+++	+	+	0
186			+	+	0	+++	+++	++	++
187			0	0	0	+++	++	++	+
	Then—→Regression								
40		1	0	0	0	++++	+	+	++
87		1	++++	++++	++	++	++++	0	+
41		2	0	+	0	+++	++	+	++
88		2	+	+	+	++	+	+	+
89		2		++++	+	+	++++	+	0
42		4	0	0	0	++	+	+	++
90		4	++	++++	++	+++	++++	+	+
91		4	0	0	0	++	+	+	++
92		6	0	0	0	++++	++	++	++
93		6	0	0	0	+	++	0	+
195	Stock	14	0	0	0	0	+	+	0
196		14	0	0	0	0	+	+	0
197		19	0	0	+	0	+	+	+
198		19	0	0	0	++	+	+	+
202		19	0	0	0	0	+	0	0
203		19	0	0	0	0	+	+	0
43	Stock + 0.25% dehydrocholic acid	1	0	0	0	+	+	+	++
94		1	++++	++++	++++	++	++++	+	++
44		2	0	0	+	+++	+	++	+
95		2	++++	0	0	+	++++	+	+
96		2	0	0	0	+	0	0	+
45		4	0	0	0	+++	++	++	+
97		4	0	0	0	0	+	0	+
99		4	++	++	0	++	++++	+	++
101		6	0	0	0	+	+	0	+
102		6	0	+	0	++	+	+	+
188		14	0	+	0	+	0	0	0
189		14	0	0	0	0	+	0	+
190		19	0	0	0	+	++	+	+
191		19	0	0	0	0	+	+	+
192		19	0	0	0	0	+	+	+
193		19	0	0	0	0	+	+	0

surface. One animal (Rabbit 198) had an impacted stone, and about it the mucosal folds were exaggerated.

Histology.—The severest lesions of the fundus of the gallbladder were seen in those animals killed at the end of four weeks of 1% dihydrocholesterol feeding. These consisted of thickening and adhesions of the mucosal folds, with formation of Aschoff-Rokitansky sinuses, fibrosis, and cellular infiltration of the lamina propria and serosa by lymphocytes and histiocytes. When the dihydrocholesterol regimen was stopped and the animals were maintained with the basal diet or basal diet plus 0.25% dehydrocholic acid for 1 to 19 weeks, the cellular infiltration diminished during the regression period. At the end of six weeks' regression all four animals still showed a mild to moderate fibrosis in the fundus of the gallbladder (Figs. 1, 2, and 3). At the end of 14 or 19 weeks' regression, Aschoff-Rokitansky sinuses persisted in all of the animals, but the fibrosis appeared less severe and the cellular infiltration was either minimal or absent (Fig. 4). The changes in the intra- and extrahepatic ducts resembled those observed in the fundus. No differences were apparent between the group fed dehydrocholic acid and the animals given the stock diet.

When the total number of plus signs (obtained as described in the Methods section and listed in Table 4) was added and averaged for the animals of each regression group, an estimate of the average pathologic involvement was obtained. Figure 5 illustrates the change of the pathologic involve-

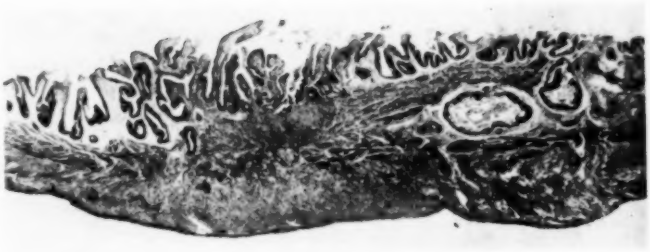

Fig. 1.—Gallbladder of Rabbit 85, killed after four weeks of dihydrocholesterol feeding. The section is taken through an exaggerated fold which encased a large concrement. The gallstones present in the fundus had a dry weight of 372 mg. Note the thickness of the wall due to hypertrophy of the muscularis and increase of fibrous tissue in the lamina propria and serosa. Aschoff-Rokitansky sinuses are seen penetrating the serosa. Hematoxylin and eosin; reduced 18% from mag. × 20.

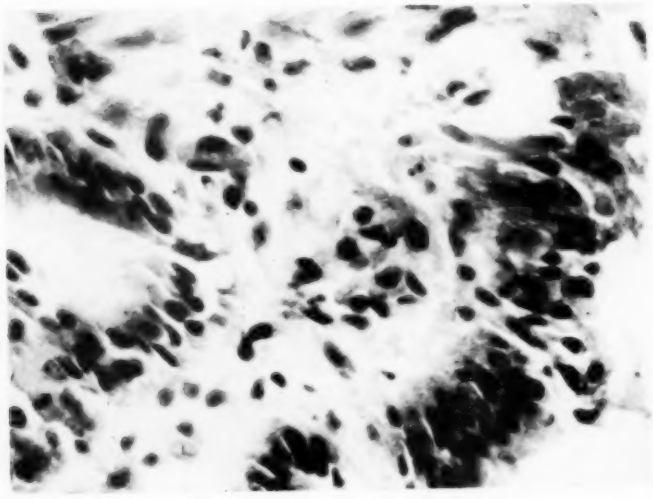

Fig. 2. — High-power view of the mucosal folds seen in Figure 1. The cells are predominantly lymphocytes and histiocytes. Hematoxylin and eosin; reduced 18% from mag. × 700.

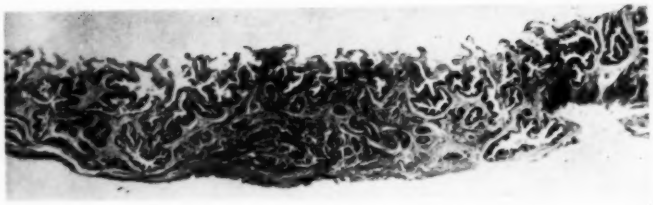

Fig. 3.—Gallbladder of Rabbit 92, fed dihydrocholesterol for four weeks then given basal diet for six weeks. Calculi weighing 430 mg. (dry weight) were present in the fundus. Hypertrophy of the muscularis as well as Aschoff-Rokitansky sinuses and increased fibrosis persist. Cellular infiltration was distinctly less, being absent in the majority of the mucosal folds. Hematoxylin and eosin; reduced 18% from mag. × 20.

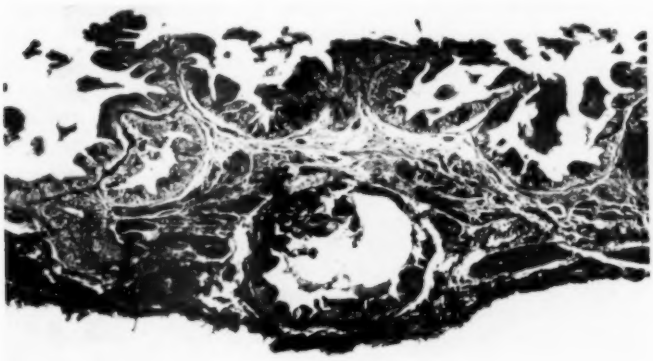

Fig. 4.—Gallbladder of Rabbit 193, fed dihydrocholesterol for four weeks then given basal diet plus 0.25% dehydrocholic acid for 19 weeks. No calculi were present in fundus or bile ducts. Aschoff-Rokitansky sinuses persist and occasionally still contain concrements. Minimal scarring of the wall is still present. Hematoxylin and eosin; reduced 18% from mag. ×90.

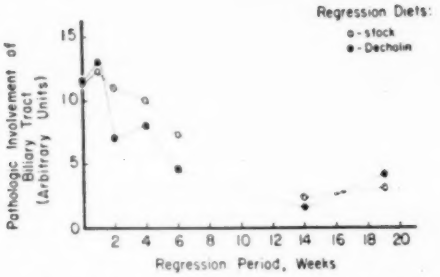

Fig. 5.—Regression of gross and microscopic lesions of biliary tract in rabbits.

ment with time during the regression experiment. By the method of grading chosen, a normal rabbit would receive a rating of 0, while the maximum of 28+ would be encountered only in rabbits which had received 1% dihydrocholesterol for about 16 weeks.* In the present experiment the animals received dietary dihydrocholesterol for only four weeks, and the 10 animals killed at the end of that period (Group C) gave an average pathologic involvement of 11.5+ (range, 8+ to 20+).

As noted previously in connection with the tissue sterol data, the severity of the disease was greatest after one week's "regression." None of the animals had fully returned to normal at the end of 19 weeks (range, 1+ to 5+). This was largely due to residual fibrotic areas in the biliary tract.

* Bevans, M., and Mosbach, E. H.: Unpublished observations.

Comment

The results of this experiment demonstrate that in the rabbit experimentally induced cholecystitis and cholelithiasis regress within 14 to 19 weeks after the causative agent (dihydrocholesterol) has been removed from the diet. The data do not enable us to decide whether the inflammatory reaction of the gallbladder receded before removal of the calculi from the fundus took place. Although the average pathologic involvement of the biliary tract decreased from about 13+ to 6+ during Weeks 1 to 6, this was largely due to the disappearance of calculi from the *bile ducts* (Table 4). The weight of the stones in the *fundus* of the gallbladder and the cellular reaction of the biliary tract remained constant during this period. It is evident, however, that the presence of calculi in the gallbladder in itself did not cause an *increase* in the inflammatory lesions of the gallbladder.

From time to time the literature contains reports on the spontaneous disappearance of human gallstones.[4] Two possible mechanisms may be involved in explaining the removal of gallstones from the gallbladder of human beings or experimental animals: 1. The gallstones dissolved. 2. The gallstones were expelled from the gallbladder after fragmentation.

In the rabbit the dissolution of the calculi is more likely to occur than in man because rabbit gallstones consist largely of bile salts,

which are potentially much more soluble in aqueous media than cholesterol or bile pigments, the major constituents of human gallstones. Nevertheless, Christensen, Dam, and Kristensen [5] have observed that in hamsters the cholesterol component of gallstones dissolves more rapidly than the bile-pigment nucleus, provided certain changes are made in the character of the diet.

During the first six weeks of the regression period the addition of dehydrocholic acid to the diet appears to accelerate the removal of dihydrocholesterol from the tissues (Table 3) and to favor a more rapid diminution of the pathologic involvement (Fig. 5). However, since each regression group consisted of only two to four animals, this impression could not be confirmed by statistical analysis of the data. Further studies will be required to decide whether this triketocholanic acid or other choleretic agents can exert significant effects on the rate of regression of the experimental cholelithiasis.

Summary

In rabbits in which cholecystitis and cholelithiasis had been produced by feeding dihydrocholesterol the biliary tract returned toward normal form and function within 14 to 19 weeks after removal of the causative agent from the diet. It could not be shown that the rate of this regression was influenced significantly by the administration of dehydrocholic acid during the regression period.

The dihydrocholesterol used in this study was supplied by the Schering Corporation. The dehydrocholic acid was given by the Ames Co., Inc. D. Harris provided technical assistance.

Columbia University Research Service, Goldwater Memorial Hospital, Welfare Island (17).

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Hypophyseal Changes in Hypothyroidism Induced by Radioactive Iodine in Man

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In a previous study of the hypophyses of 27 patients with thyroid disease,[1] hyperplasia of sparsely granulated amphophilic cells in patients with spontaneous thyroid deficiency was noted. For several years it has been recognized that thyroid deficiency induced by radioactive iodine in mice may be followed not only by hypophyseal hyperplasia but by actual tumors composed of cells morphologically similar to those which increase in human myxedema.[2,3] Although radioactive iodine had been administered to six of the patients previously reported, the circumstances of its use were such that no conclusions could be drawn as to its effects in man. The present paper describes the hypophyses of an additional 27 patients, 12 of whom had received radioactive iodine. In comparing their hypophyses with those of patients of similar thyroid status who had not received this drug, certain conclusions as to the morphological effects of thyroid deficiency induced by radioactive iodine in man now seem possible.

Material and Methods

Hypophyses were studied by the method previously described.[1] If whole glands were available, they were weighed on a torsion balance after formalin fixation and removal of the stalk and membranes. Differential counts were performed on sections stained by the periodic acid-Schiff reaction and orange G. Cell types were classified as follows.

1. *Normal Basophils.*—Densely granulated intensely Schiff-positive cells (Figs. 2 and 3).

2. *Acidophils.*—Densely granulated Schiff-negative cells staining brightly with orange G (Figs. 3 and 6).

3. *Amphophils.*—Relatively sparsely granulated weakly Schiff-positive cells varying considerably in morphology, numbers of granules, and intensity of staining reaction. The cell boundaries are irregular and indistinct as opposed to the sharp rounded outlines of the conventional chromophils. The nucleus is often large and vesicular, frequently with a prominent nucleolus. Occasional large, intensely Schiff-positive droplets may occur in the cytoplasm (Figs. 2, 4, and 5).

4. *Hypertrophic Amphophils.*—Cells derived from the amphophils by enlargement of the nucleus and loss of cytoplasm and granules.

5. *Chromophobes.*—Cells with small nuclei and a relatively small amount of cytoplasm in which no granules can be demonstrated histologically.

6. *Hyaline Basophils or Crooke's Cells.*—Basophils in which part or all of the granulated cytoplasm has been replaced by opaque hyaline Schiff-negative material.

Endocrine glands other than the hypophysis were weighed fresh and studied in hematoxylin-and-eosin sections. Pertinent clinical and anatomic data on these patients are summarized in Tables 1 to 6.

Results

Thyroid Deficiency.—The material included six patients with primary thyroid deficiency due to spontaneous thyroiditis and fibrosis who had not received radioactive iodine (Table 1). Only one patient (Case 1) had received no replacement therapy. Unlike the three patients with untreated myxedema reported previously,[1] this man did not have hypophyseal enlarge-

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TABLE 1.—*Endocrine Glands in*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid	
					Weight, Gm.	Description
1	M	67	Coronary thrombosis; chronic fibrosing thyroiditis	None	31	Fibrosis, masses of lymphocytes; rare acini with nuclear atypicality
2	F	62	Myxedema heart disease	Triiodothyronine, 5 mg/day, 4 days	5	Severe fibrosis; rare nodules composed of tiny acini
3	F	71	Bronchopneumonia; myxedema; myocardial infarction, healed	Thyroid, 15 mg/day, 3 wk.	3	Masses of lymphocytes; small atypical acini with giant nuclei
4	M	77	Myocardial infarction; myxedema (PBI 1.8 μg.%)	Thyroid, 20-60 mg/day, 7 yr. None for at least 3 days	11	Fibrosis, lymphocytes, rare atypical acini
5	F	60	Bronchopneumonia; myxedema; myocardial infarction, healed	Thyroid, 15 mg/day plus Triiodothyronine, 50 mg/day, 9 days	14.5	Severe fibrosis; rare lymphocytes & nests of squamous epithelium
6	F	70	Myocardial infarction; treated myxedema	Thyroid, 100 mg/day, 5 mo.	8.5	Fibrosis, focal lymphocytes; small acini with severe focal nuclear atypicality

* Ad indicates adenoma; Amp., amphophils; HA, hypertrophic ampophils; Ac, acidophils; NB, normal basophils; Chr, chromophobes, and Hy B, hyaline basophils. The last 6 are given as relative percentages.

ment. However, his gland did contain a microscopic adenoma composed predominantly of amphophils similar to that shown in Figure 1. On differential count, amphophils comprised nearly 40% of the cells present and hypertrophic amphophils were also relatively abundant (Fig. 2). Basophils were within normal limits, and acidophils were somewhat reduced. In the patients receiving thyroid therapy (Patients 2 to 6) the percentage of amphophils tended to fall in direct relation to the adequacy of treatment and hypophyseal weight tended to decrease. There was no systematic effect on the basophils, and the acidophils tended to rise. In Patient 6, who was clinically euthyroid, the differential count was normal except for a slight excess of acidophils (Fig. 3). These findings support the suggestion made previously that the amphophils may be the source of thyrotropic hormone in man.

Five initially euthyroid patients had been treated with radioactive iodine (Table 2). One (Patient 7) had received the drug in an unsuccessful attempt to improve a Parkinsonian tremor. Three (Patients 8 to 10) had been treated for coronary artery disease, and one (Patient 11), for rheumatic heart disease. Two patients (7 and 8)

Fig. 1.—Microscopic amphophil adenoma in the hypophysis of Patient 21, a 59-year-old woman who had total thyroidectomy 18 years previously, acid hematein; × 37.

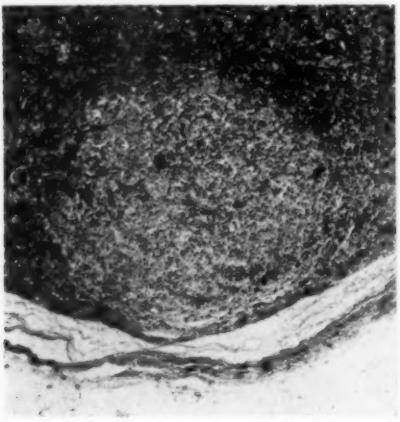
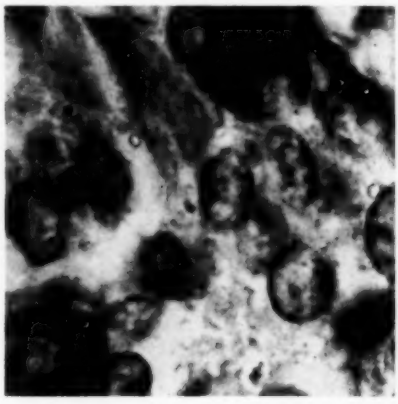


Fig. 2.—Hypophysis of Patient 1, a 67-year-old man with spontaneous untreated myxedema. Most of these cells are amphophils with large vesicular nuclei, sparsely granulated cytoplasm, and indistinct cell boundaries. The two very dark cells are basophils. Periodic acid-Schiff orange G; × 950.



Patients with Thyroid Deficiency

Cells Counted	Wt., Gm.	Hypophysis * Ad	Amp	HA	Ae	NB	Chr	HyB	Other Abnormalities
2,685	0.50	+	38.2	2.3	21.6	8.2	29.7	0	Severe testicular atrophy with persistent Leydig cells; nuclear atypicality in adrenals
5,749	0.66	0	17.1	1.8	18.3	8.3	54.5	0	Severe ovarian atrophy
2,414	0.57	0	18.2	1.4	33.5	15.9	31.0	+	Diabetes mellitus
3,845	0.46	0	15.6	1.4	37.6	21.6	23.8	+	Severe testicular atrophy
2,650	Not weighed	0	8.1	1.2	30.2	15.0	45.5	0	Diabetes mellitus; hirsutism
3,356	0.43	0	6.9	0.8	46.2	9.3	36.8	0	Bilateral oophorectomy, 32 yr.

were clinically myxedematous at the time of death. Both had hypophyseal enlargement and amphophil hyperplasia (Fig. 4). These findings were less marked in Patient 8, probably because he had received small amounts of supplementary thyroid during his last month of life. In the three patients who died before they became clinically myxedematous, the hypophysis was smaller and the percentage of amphophils much lower. None of these hypophyses showed anatomic signs of radiation damage, and none contained discrete adenomas. Histologic examination failed to show any consistent differences between the hypophyses of patients with spontaneous myxedema and those in whom myxedema had been induced by I^{131} (compare Figs. 2 and 4). The morphological reaction of the human hypophysis would, therefore, appear to be the same in both conditions.

Thyrotoxicosis.—Three patients had a history of thyrotoxicosis treated by other means than radioactive iodine (Table 3). Patient 12 had had a subtotal thyroidectomy 30 years previously and died of coronary

Fig. 3.—Hypophysis of Patient 6, a 70-year-old woman with spontaneous adequately treated myxedema. No amphils are seen. Two dark basophils are surrounded by pale gray acidophils. Note comparatively small size of nuclei, well-granulated cytoplasm, and distinct cell boundaries. Periodic acid-Schiff orange G; × 950.

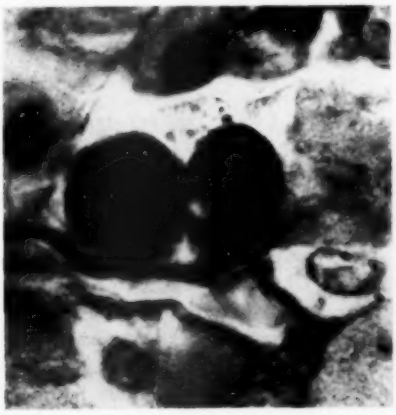


Fig. 4.—Hypophysis of Patient 8, a 64-year-old man who had received 30 mc. of I^{131} one year previously for heart disease and was clinically myxedematous. Note abundant amphophils and resemblance of these cells to those seen in spontaneous myxedema, Figure 2. Periodic acid-Schiff orange G; × 950.

TABLE 2.—*Endocrine Glands in Patients with*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid	
					Weight, Gm.	Description
7	F	48	Parkinson's disease; myxedema	33 mc. I^{131}, 2 yr.	3.5	Moderate fibrosis; many large atypical nuclei in small acini
8	M	64	Coronary artery disease; myxedema	29.8 mc. I^{131}, 1 yr. Thyroid, 15-20 mg./day, 4 wk.	8.0	Severe fibrosis with occ. lymphocytes; nuclear atypicality & pleomorphism
9	M	49	Coronary artery disease (PBI 6.6 μg.%)	20 mc. I^{131}, 5 mo.	19	Moderate fibrosis; small, histologically normal acini
10	M	49	Coronary artery disease (PBI 10.3 μg.%)	75 mc. I^{131}, 1 mo.	13	Hemorrhage, fibrosis, granulation tissue, few lymphocytes & P. M. N.; large areas of normal thyroid
11	M	26	Rheumatic heart disease (PBI 5.5 μg.%)	30 mc. I^{131}, 3 wk.	19	Focal fibrosis; rare necrotic areas with P. M. N. & lymphocytes; most of tissue normal

* See Table 1 for explanation of abbreviations.

TABLE 3.—*Endocrine Glands in*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid	
					Weight, Gm.	Description
12	F	72	Myocardial infarct (thyrotoxicosis)	Subtotal thyroidectomy, 30 yr.	Rt. lobe, 8.2 Lt. lobe, 9.4	Fibrotic Small active acini
13	M	65	Glioma; meningitis (thyrotoxicosis)	Subtotal thyroidectomy, 23 yr.	5.5	Histologic signs of moderate activity
14	M	72	Cerebrovascular accident; mesenteric artery thrombosis; (thyrotoxicosis)	Propylthiouracil, 100-300 mg./day, 7 yr. None for at least 2 wk.	120	Acini mostly normal; rare foci of activity & severe nuclear atypicality

* See Table 1 for explanation of abbreviations.

TABLE 4.—*Endocrine Glands in Patients with*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid	
					Weight, Gm.	Description
15	M	79	Coronary thrombosis (thyrotoxicosis); treated myxedema after I^{131}	Propylthiouracil in past; 30 mc. I^{131}, 10 yr. Thyroid, 100 mg/day, irregularly, 1 yr.	6	Severe fibrosis, few lymphocytes; extreme nuclear atypicality
16	F	72	Cerebrovascular accident (thyrotoxicosis); treated myxedema after I^{131}	Subtotal thyroidectomy, 27 yr.; 1 course propylthiouracil, 10 yr. 12 mc. I^{131}, 8 yr.; thyroid, 60 mg/day, 7 ½ yr.		Not identified grossly; microscopic examination shows fibrosis, occasional lymphocytes, moderate nuclear atypicality
17	M	64	Auricular fibrillation; pulmonary edema; thyrotoxicosis (PBI 8.3 μg.%)	2 courses propylthiouracil, ages 56 and 61; 9.0 mc. I^{131}, 4 mo.	41	Slight focal fibrosis; histology otherwise normal

* See Table 1 for explanation of abbreviations.

thrombosis before her terminal thyroid status could be determined. She had a large hypophysis containing numerous amphophils. Patient 13 had had a subtotal thyroidectomy 23 years before death. His terminal basal metabolic rate was +25, but his radioactive iodine uptake was normal. Fewer amphophils were present. Patient 14 was a 72-year-old physician who had maintained himself in a euthyroid state for seven years before death by doses of propylthiouracil ranging from 100 to 300 mg. per day. Although his hypophysis was only slightly enlarged and his amphophils but little increased, he did have a microscopic amphophil adenoma.

Three persons had received radioactive iodine for thyrotoxicosis (Table 4). Patient 15 survived 10 years after receiving 30 mc. and was clinically myxedematous much of that time. During his terminal year of life he received small amounts of thyroid

Thyroid Deficiency Treated with Radioactive Iodine

		Hypophysis *							
Cells Counted	Wt., Gm.	Ad	Amp	HA	Ac	NB	Chr	HyB	Other Abnormalities
2,645	0.80	0	32.1	1.7	22.8	10.7	32.7	+	
2,240	0.66	0	25.4	2.3	35.9	20.8	15.6	+	
3,787	0.55	0	8.9	2.8	14.3	10.3	63.7	0	
2,360	0.44	0	11.5	2.1	26.5	28.6	31.3	+	
3,423	0.36	0	11.9	1.2	30.4	12.4	44.1	+	

Patients with Thyrotoxicosis

		Hypophysis *							
Cells Counted	Wt., Gm.	Ad	Amp	HA	Ac	NB	Chr	HyB	Other Abnormalities
2,606	0.70	0	29.8	1.2	43.2	22.6	12.2	0	
2,585	Not weighed	0	14.3	1.4	31.1	26.8	25.1	1.3	
2,324	0.60	+	10.8	1.7	40.1	9.8	37.6	0	

Thyrotoxicosis Treated with Radioactive Iodine

		Hypophysis *							
Cells Counted	Wt., Gm.	Ad	Amp	HA	Ac	NB	Chr	HyB	Other Abnormalities
3,106	0.54	+	27.6	2.6	30.3	9.7	29.8	+	
3,720	0.96	0	18.3	2.0	25.5	21.7	32.5	+	
2,328	0.55	0	10.9	0.8	41.5	16.1	30.7	+	Bilateral testicular atrophy following herniorrhaphy, age 15, and gonorrhea, age 25; decreased body hair

hormone. His hypophysis was not significantly enlarged but did contain a small amphophil adenoma and showed striking amphophil hyperplasia. Patient 16 had myxedema induced by 12 mc. of I^{131} eight years previously and had been receiving small doses of thyroid for seven and one-half years. She had an unusually large hypophysis, with moderate amphophil hyperplasia. Patient 17 had received 9 mc. of I^{131} four months before death and was still mildly toxic. His hypophysis was of normal size; his amphophils were much lower, and no adenoma was present.

As in Patients 7 to 11, the findings in these patients with thyrotoxicosis suggest that the morphology of the hypophysis at the time of death correlates with the terminal thyroid status of the patient rather than with the amount of radioactive iodine administered.

Carcinoma of the Thyroid.—Six patients with a past history of carcinoma of the thyroid had received no I^{131} (Table 5).

TABLE 5.—*Endocrine Glands in*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid
18	M	89	Ca thyroid with massive metastases	None	Totally replaced by tumor
19	M	58	Ca thyroid with massive metastases	None	Areas of normal thyroid interspersed with well-differentiated carcinoma
20	F	76	Ca thyroid with metastases	None	Left lobe replaced by tumor; right lobe 5 gm.; fibrosis, lymphocytes, atypical nuclei
21	F	59	Vertebral artery thrombosis (Ca thyroid)	Total thyroidectomy, 18 yr.; thyroid, 100 mg/day, irregularly, 18 yr.	Surgically absent, 18 yr.
22	F	35	Hürthle cell Ca thyroid with massive metastases	Hemithyroidectomy, 8 yr.; total thyroidectomy, 5 yr. Testosterone+estrogens in past; thyroid, 60 mg/day, terminally	Surgically absent, 5 yr.; massive metastases
23	M	43	Rheumatoid arthritis; bronchopneumonia (Ca thyroid)	Hemithyroidectomy, 5 yr.; cortisone+corticotropin (ACTH) in past; methyltestosterone, 10 mg/day terminally	Right lobe surgically absent; left lobe 19 gm.; diffuse fibrosis; focal activity

* See Table 1 for explanation of abbreviations.

TABLE 6.—*Endocrine Glands in Patients with*

Patient	Sex	Age	Diagnoses	Treatment	Thyroid
24	M	71	Ca thyroid with massive metastases	Hemithyroidectomy 20 yr. (fetal adenoma) Total thyroidectomy 10 yr. (carcinoma); 1,289 mc. I^{131}, 8 yr.; propylthiouracil, methimazole (Tapazole), & thyroid in past Retained iophendylate (Pantopaque), 2 yr.; 50 mg. triiodothyronine, 2 days	Surgically absent, 10 yr.; massive metastases
25	F	65	Acute cholecystitis; Ca thyroid with metastases; Ca breast with metastases	Total thyroidectomy 5 yr.; 763 mc. I^{131}, 4 ½ yr.; propylthiouracil, testosterone, & thyroid in past; no recent hormones	Surgically absent 5 yr.; metastases
26	F	60	Hürthle-cell Ca thyroid with massive metastases; adenocarcinoma cecum	Hemithyroidectomy 11 yr.; total thyroidectomy 6 yr.; 547 mc. I^{131}, 5 yr.; estrogens in past, none for 2 yr.; thyroid, 100–200 mg/day, 4 ½ yr.	Surgically absent 6 yr.; metastases
27	F	78	Squamous Ca thyroid with tracheal obstruction	30 mc. I^{131}, 3 mo.; diethylstilbestrol, 1 mg/day plus saturated KI, 15 drops/day, 3 mo. to 3 wk. before death; hemithyroidectomy, 3 wk.	Left lobe surgically absent; right lobe replaced by Ca

* See Table 1 for explanation of abbreviations.

Three, in fact, (Patients 18 through 20), had received no treatment of any sort. One patient (Patient 21) had had a total thyroidectomy 18 years previously and had taken thyroid irregularly to control the resultant myxedema. Two patients (Patients 22 and 23) had had hemithyroidectomies and had received moderate amounts of various hormones. Three of these hypophyses were found to be enlarged (Patients 18, 19, and 22); one contained a microscopic amphophil adenoma (Patient 21, Fig. 1), and all six showed hyperplasia of amphophils and hypertrophic amphophils, suggesting, but again not proving, that considerable thyrotropic hormone was being produced.

Four patients with carcinoma of the thyroid had received radioactive iodine (Table 6). Patient 24 had been given a total of 1289 mc. over the last eight years of life and had had a total thyroidectomy 10 years before death. He had several episodes of clinical myxedema during the course of his disease. However, he had also been treated periodically with thyroid hormone and for two years before death had elevated serum protein-bound iodine levels as a result of iodine-containing dye retained in his spinal canal following a myelogram. At the time of death his hypophysis was small and contained no adenomas. His amphophils were moderately elevated on differential count

Patients with Carcinoma of the Thyroid

Hypophysis *									
Cells Counted	Wt., Gm.	Ad	Amp	HA	Ac	NB	Chr	HyB	Other Abnormalities
2,264	0.74	0	19.8	2.1	40.4	9.4	28.3	+	Chondroma larynx Paget's disease of bone
2,604	0.68	0	15.1	2.1	29.4	23.2	30.2	0	
3,558	Not weighed	0	15.8	1.4	25.7	30.8	26.3	+	Rt. ovary surgically absent; hyperostosis frontalis interna
2,353	Not weighed	+	20.4	2.3	35.4	11.0	30.9	+	
3,471	0.74	0	20.2	1.8	43.1	6.1	28.8	0	Goiter for 16 yr., onset during pregnancy 4 yr. amenorrhea after x-ray to spine
3,138	0.53	0	15.0	3.5	34.7	11.8	35.0	+	

Carcinoma of the Thyroid Treated with Radioactive Iodine

Hypophysis *									
Cells Counted	Wt., Gm.	Ad	Amp	HA	Ac	NB	Chr	HyB	Other Abnormalities
1,284	0.40	0	17.8	2.0	16.7	26.5	37.0	+	Diabetes mellitus, 10 yr.
3,373	0.66	0	15.7	1.5	30.2	17.8	34.8	+	3 cm. adrenal adenoma
5,533	0.72	+	8.6	0.7	47.0	5.0	38.7	0	Hysterectomy for dysmenorrhea, age 25
3,063	0.64	0	6.1	2.2	32.9	20.4	38.4	+	

but were no higher than in the three patients with untreated carcinoma of the thyroid, all of whom were clinically euthyroid at the time of death.

Patient 25 had had a total thyroidectomy and had received 763 mc. of I^{131}. She had also received some thyroid hormone and some testosterone for palliation of an independent carcinoma of the breast but had been given no hormones for several months before death. Her hypophysis was slightly above average weight, and no adenomas were found. Her amphophils were increased to approximately the same degree as in Patient 24 (Fig. 5).

Patient 26 had also been treated by total thyroidectomy and 547 mc. of radioactive iodine, after which she became severely myxedematous. However, for the last months of life she had been maintained with large doses of thyroid hormone. At her death from an independent carcinoma of the cecum the differential cell count in her hypophysis was virtually normal except for a slight excess of acidophils (Fig. 6). However, the gland was large, and a microscopic adenoma was present.

Patient 27 had received 30 mc. of I^{131} only three months before death and had been given 1 mg. of diethylstilbestrol plus

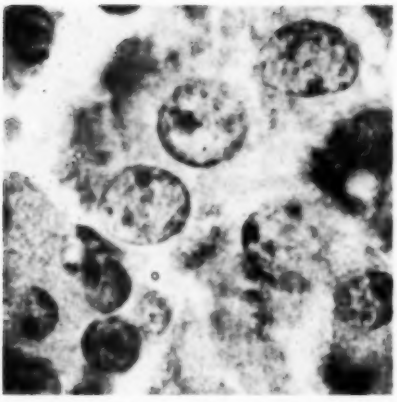


Fig. 5.—Hypophysis of Patient 25, a 65-year-old woman with carcinoma of the thyroid who had received 763 mc. of I^{131} four and one-half years previously. No recent hormone treatment. Note abundant amphophils and resemblance of these cells to those in Patient 8, Figure 4, who had received only 30 mc. of I^{131}, and in Patient 1, Figure 2, who had received no I^{131}. Periodic acid-Schiff orange G; × 950.

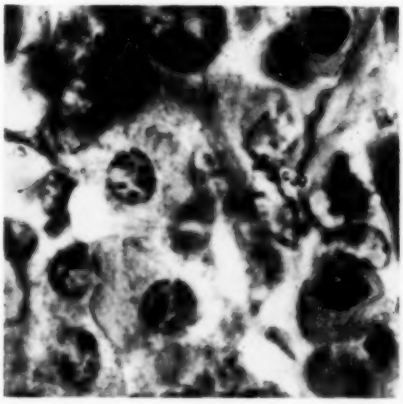


Fig. 6.—Hypophysis of Patient 26, a 60-year-old woman who had received 547 mc. of I^{131} five years previously and had been given 100 to 200 mg. of thyroid per day subsequently. Note relative scarcity of amphophils and abundance of pale gray acidophils with small nuclei and distinct cell boundaries. Periodic acid-Schiff orange G; × 950.

15 drops of saturated potassium iodide solution each day. Her amphophils were also in the normal range, although both hypertrophic amphophils and basophils were unusually abundant.

Interpretation of the results in carcinoma of the thyroid is difficult, since the histological findings at the time of death are the resultant of many factors—the patient's disease, thyroid surgery, radioactive-isotope therapy, and the administration of several types of hormones. However, there is no indication that I^{131} affected hypophyseal morphology in any of these patients by any mechanism other than by change in thyroid status. Patients 26 and 27 would appear to demonstrate that exogenous hormone or iodide medication can suppress an expected amphophil hyperplasia even in the presence of radioactive iodine therapy.

Histology of the Thyroid.—Unlike the hypophyses, the thyroids of patients receiving I^{131} did show changes which might be ascribed to the direct action of radiation. In glands of patients dying within a month after administration of the drug there were focal areas of necrosis, granulation tissue, and inflammatory cells, both lymphocytes and polymorphonuclear leukocytes. With increasing periods of survival, fibrosis tended to increase and scattered foci of lymphocytes occurred in the connective tissue. The total thyroid weight also tended to decrease.

In patients surviving a year or more after receiving I^{131}, there were giant bizarre hyperchromatic nuclei in many of the thyroid follicles. While it is entirely possible that this nuclear change may have been a direct result of radiation, it is also possible that other factors, such as hypophyseal stimulation, may have been involved. Microscopic areas showing this change were also found in the thyroid of Patient 14, a man who had been given propylthiouracil for seven years and had received no radiation of any sort. Occasional hypertrophic atypical nuclei were also seen in thyroids of myxedematous patients who had received neither I^{131} nor propylthiouracil but whose thyroids may well have been subjected to increased thyrotropic hormone levels for long periods of time.

While these nuclear changes resemble those which may be seen in carcinoma, it should be emphasized that no discrete tumors, either benign or malignant, were

found in the thyroids of any of these patients which could be ascribed either to radioactive iodine or to goitrogenic drugs. Similar nuclear pleomorphism following I^{131} therapy has been observed by others.[4,5]

Comment

Including the *27* cases previously reported from this laboratory, the hypophyses of 54 patients with thyroid disease have now been studied by a uniform quantitative method. The results suggest that the human hypophysis, like that of the mouse, may react to thyroid deficiency by increase in weight, by hyperplasia of a specific cell type, and, possibly, by adenoma formation. However, all three of these phenomena do not necessarily occur at once. These changes suggest that the specific cell type involved, the amphophil, may be the source of thyrotropic hormone.

Since microscopic hypophyseal adenomas such as occurred in many of these patients are very common in routine autopsy material,[6] there is no proof that they are the result of thyroid deficiency. However, the fact that the cells of which they are composed are morphologically identical with those which clearly increase in thyroid deficiency suggests that thyroid deficiency may be one factor in their pathogenesis.

The concept that thyroid deficiency may be responsible for some hypophyseal adenomas in man actually antedates the use of radioactive iodine by many years. For instance, a large so-called chromophobe adenoma occurring in a 26-year-old woman with congenital thyroid aplasia was reported by Schultze, in 1914, as an illustration of Erdheim's theory of hypophyseal enlargement with tumor formation in response to a primary end-organ deficiency.[7] When amphophils, occurring either in mice or in human patients, are stained by many of the routine methods, they appear to be relatively agranular cells and may be classified as "chromophobes."[2] It seems more than possible that some of the so-called chromophobe adenomas in man are not the inert lesions popularly described in textbooks but that they may have an endocrine significance comparable to the experimental hypophyseal tumors which have been induced in laboratory animals.

The histological changes observed in the hypophyses of these patients may also be pertinent to the treatment of thyroid tumors. Thus it has been reported that while induced thyroid deficiency (which causes amphophil hyperplasia) may increase the uptake of I^{131} by metastatic thyroid carcinoma, it may also act as a growth stimulus to metastases. Desiccated thyroid (which decreases the percentage of amphophils) has been found of value in such patients.[8] If changes in the amphophils parallel changes in the secretion of thyrotropic hormone, they may be the anatomic explanation of this change in clinical status.

Summary

The hypophyses of 12 patients who had received from 9 to 1289 mc. of I^{131} from 3 weeks to 10 years before death were compared with the hypophyses of 15 patients of similar thyroid status who had not received radioactive iodine. In both groups clinical thyroid deficiency tended to increase hypophyseal weight and the relative percentage of sparsely granulated amphophilic cells. In both groups the histological effects of myxedema appeared to be reversed by thyroid or iodide therapy. The changes produced by I^{131} were related to the thyroid status of the patient but not to the total amount of drug administered. In no case was there any anatomic evidence of radiation damage to the hypophysis.

The thyroids of patients dying within a month after I^{131} treatment showed focal radiation necrosis. In those surviving longer periods, the thyroid tended to decrease in weight and to show increased fibrosis and lymphocytes. Giant hyperchromatic nuclei appeared; however, these were also found in one patient given propylthiouracil and in several with spontaneous myxedema, suggesting that they were secondary to hypo-

physeal stimulation rather than to radiation per se.

It was concluded that the hypophyseal amphophils are the most probable source of thyrotropic hormone. The possibility that thyroid deficiency may have produced microscopic hypophyseal adenomas in a few cases and a possible relation of hypophyseal changes to growth of thyroid tumors were discussed.

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Tissue Reactions to Autologous and Homologous Musculofascial Grafts

I. Effect of Exposure of Grafts in Vitro to X-Radiation Before Transplantation

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Introduction

Studies of host-graft tissue interactions involving musculofascial autografts in rabbits revealed orderly sequences of degeneration and organization of the grafts. Under the same conditions musculofascial homografts showed similar sequences modified by development of an inflammatory reaction, impairment of fibroblastic proliferation, and reduction of collagen deposition.[3] In subsequent experiments musculofascial grafts were exposed to extremes of low and high temperatures in vitro prior to implantation. This modified the interactions so that autografts and homografts elicited the same type of response. This was characterized by avascular collagenous encapsulation of the grafts by the host. It seemed that conditions which decreased admixture of proliferating tissues of the host with those of the graft reduced the intensity and specificity of classical host-graft interactions.[1]

In view of the observed effects of heat and cold, an experiment was designed to study the pattern of host-graft interactions when transplants had been subjected to x-irradiation in vitro before surgical implantation in the host. This was carried out with the hope that a dosage might be found at which factors in grafts responsible for undesirable host-homograft reactions might be destroyed without disturbing the ability of the grafts to survive.

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Method

Musculofascial transplants, measuring $2 \times 1.5 \times 0.5$ cm. in dimensions, were resected from rabbits as previously described.[3] The tissues for transplantation were placed in sterile Stender dishes containing isotonic saline. Each transplant was then exposed to a single dose of x-radiation ranging from 100 to 90,000 r. Control grafts were protected against radiation but otherwise handled in a similar way. A Westinghouse x-ray therapy machine was used without filter at 200 kv. and 18 ma. The dose was regulated by duration of exposure. When 90,000 r was given the temperature of the Stender dish rose 2 degrees (C). At lower doses of radiation the increase in temperature was negligible. After irradiation, the tissues were implanted together with nonirradiated control autologous and homologous transplants. After one, two, or three weeks and occasionally a long period, the animals were killed by injection of 1% procaine solution into the cisterna magna. After fixation of tissues in 4% formaldehyde solution, sections of the transplants were prepared and routinely stained with hematoxylin and eosin for microscopic study.

In other experiments, a lead chamber was used to protect approximately one-half of the transplant (Fig. 1). These transplants were irradiated as previously described. This method eliminated some variables and permitted study of the control and irradiated halves of the graft in the same microscopic section (Fig. 2).

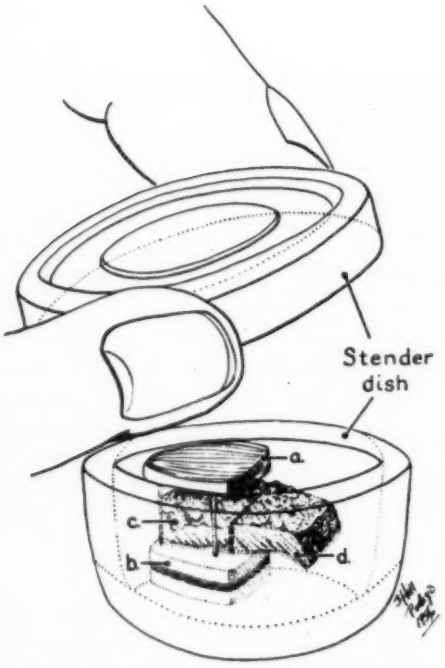
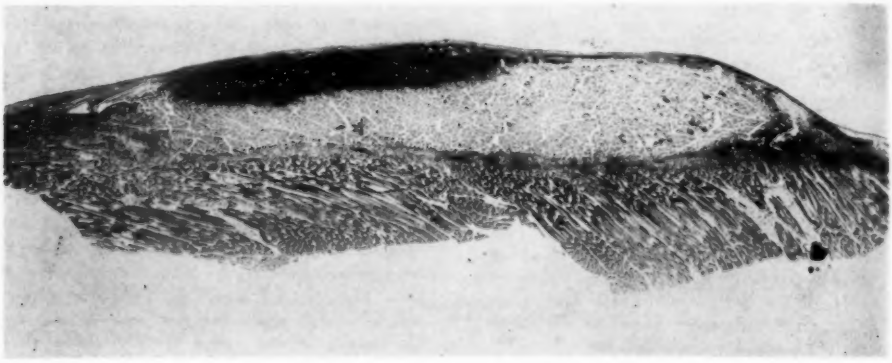


Fig. 1.—This drawing shows the method used to protect parts of musculofascial transplants from effects of x-radiation. The sterile Stender dish contains the protective chamber which occupies approximately one-half the dish. The roof of the chamber consists of a solid lead plate (*a*), 3 mm. in thickness, supported by pins. The floor of the chamber is made of two closely-approximated lead plates (*b*), with a total thickness of 6 mm. A musculofascial transplant has been inserted between the upper and lower lead plates so that one-half of the transplant (*c*) is protected and the remaining half (*d*) is exposed. After this, the chamber was irradiated, with the x-rays striking the lowermost region first.

Results

Control autologous and homologous musculofascial transplants were well healed in place by the end of the first week. A pannus of vascularized granulation tissue had grown over the fascia of each transplant. At the end of the second week microscopic examination was most informative. Each autograft showed normal sequences of degeneration, organization, and absorption. A broad musculofascial zone containing proliferating fibroblasts, multinucleated striated mesenchymal cells, and many vascular channels embedded in a collagenous matrix had formed. Each homologous musculofascial transplant, two weeks old, showed similar sequences of healing complicated by inflammation characterized by lymphocytic infiltration and angiitis, with impaired fibroblastic division and reduced collagen deposition (Fig. 3). By the end of the third or fourth week, organization and resorption had progressed to the point

Fig. 2.—This is a low-power photomicrograph of a homologous musculofascial transplant, two weeks of age. Prior to transplantation one-half of the graft was irradiated with 5000 r in vitro. The other half was shielded from radiation as shown in Figure 1. The transplant is well healed in place. The empty area above the transplant represents the usual bursal space. A delicate pannus has grown over the fascia of the transplant. In the left half of the transplant, which had been shielded, the musculofascial zone is broad and contains a dense accumulation of lymphoid cells in vascularized granulation tissue. In the right half of the transplant, which had been irradiated, the reaction failed to develop. The subjacent skeletal muscle has no unusual changes.



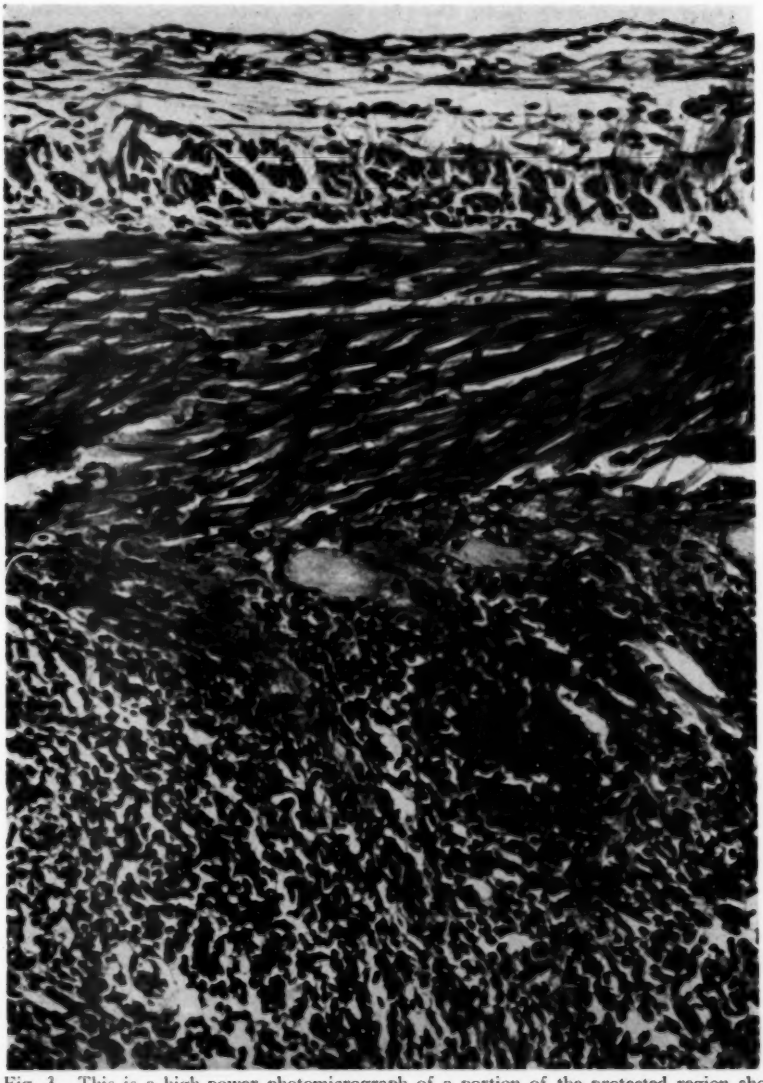


Fig. 3.—This is a high-power photomicrograph of a portion of the protected region shown in Figure 2. The empty space above the transplant represents the bursal space. A delicate vascularized pannus has grown over the fascia of the transplant. Beneath the fascia there is a broad musculofascial zone which contains vascularized granulation tissues, absorbing skeletal muscle and a massive accumulation of lymphoid cells. This type of response is characteristic of a host-homograft incompatibility reaction.

where little of either type of graft remained.

Gross examination of autologous and homologous transplants treated with 90,000 to 10,000 r prior to implantation revealed that they were well healed in place by the end of the second week. The bursal space, which customarily formed over control grafts, also developed over irradiated grafts. The fascia of each transplant was pearly white except at the periphery, where blotchy red granulation tissue was noted.

Changes in Host-Graft Tissue Interactions When Autologous and Homologous Musculofascial Tissues Received Different Amounts of X-Radiation in Vitro Before Implantation

Types of Grafts	Age of Grafts, Days	X-Ray Dose, r	Grafts, No.	Microscopic Types of Host-Graft Tissue Reaction				
				Autologous	Homologous	Indeterminate *	Other Vascularizing †	Inert ‡
Autograft	14	100	2	2	0	0	0	0
Homograft	14	100	4	0	4	0	0	0
Autograft	14	250	2	2	0	0	0	0
Homograft	14	250	7	0	7	0	0	0
Autograft	14	500	2	2	0	0	0	0
Homograft	14	500	9	0	0	9	0	0
Homograft	21	500	3	0	0	3	0	0
Homograft	14	750	8	0	0	8	0	0
Autograft	14	1,000	2	0	0	0	2	0
Homograft	7	1,000	2	0	0	0	2	0
Homograft	14	1,000	5	0	0	0	5	0
Homograft	21	1,000	2	0	0	0	2	0
Autograft	14	2,500	3	0	0	0	3	0
Homograft	14	2,500	6	0	0	0	6	0
Homograft	35	2,500	2	0	0	0	2	0
Autograft	14	5,000	2	0	0	0	2	0
Homograft	14	5,000	2	0	0	0	2	0
Autograft	14	10,000	1	0	0	0	0	1
Homograft	14	10,000	4	0	0	0	0	4
Autograft	14	20,000	3	0	0	0	0	3
Homograft	14	20,000	3	0	0	0	0	3
Autograft	14	45,000	2	0	0	0	0	2
Homograft	14	45,000	3	0	0	0	0	3
Autograft	7	60,000	2	0	0	0	0	2
Autograft	7	90,000	2	0	0	0	0	2
Autograft	14	90,000	3	0	0	0	0	3
Homograft	14	90,000	3	0	0	0	0	3

* Displayed some features of the classical reactions.
† Penetration of the graft by vascularized stroma of the host without inflammation.
‡ Fibrous encapsulation without vascular penetration of the graft.

This was in direct contrast to the pink richly vascularized appearance of the fascia of control grafts.

Microscopic study of grafts treated with 90,000 to 10,000 r revealed them to be firmly healed in place two weeks after transplantation. A thin avascular pannus had grown over the fascia of each transplant. The fascia was acellular, stained faintly, and showed no penetration by vascularized stroma of the host. Inflammation was absent. The muscle cells remained close to the overlying fascia. Absorption of the transplants was slow, with sluggish stromal penetration of the grafts at the margins of the graft bed (Table). This was designated as an inert reaction.

After a dose of 5000 r, autograft and homograft reactions were identical, but the sequences of healing described after doses from 90,000 to 10,000 r began to show a change. This consisted of vascularization of the pannus, with vessels from the host penetrating the fascia of the grafts and branching throughout the narrow musculofascial zone. In spite of rich vascularization, there was no fibroblastic proliferation, collagen deposition, or inflammatory-cell infiltration (Figs. 2 and 4). The host still seemed unable to distinguish between autograft and homograft. Degeneration and absorption of transplants were still slow, but less so than after larger doses of radiation.

After doses of 750 r and 500 r, homografts retained the capacity to elicit a focal inflammatory-cell infiltration, but this lacked the intensity of the control host-homograft reactions. The inflammatory reaction did not increase with prolongation of the age of the graft. This indicated that the effect of radiation on factors in the graft which stimulated the inflammation was not temporary. At this same dosage host-autograft reactions were similar to but less pronounced than the interactions noted when autografts received no irradiation prior to implantation.

After doses of 250 r and 100 r, typical homologous and autologous reactions similar to those elicited by unirradiated control grafts were encountered.

The results described above were substantiated under closely controlled condi-

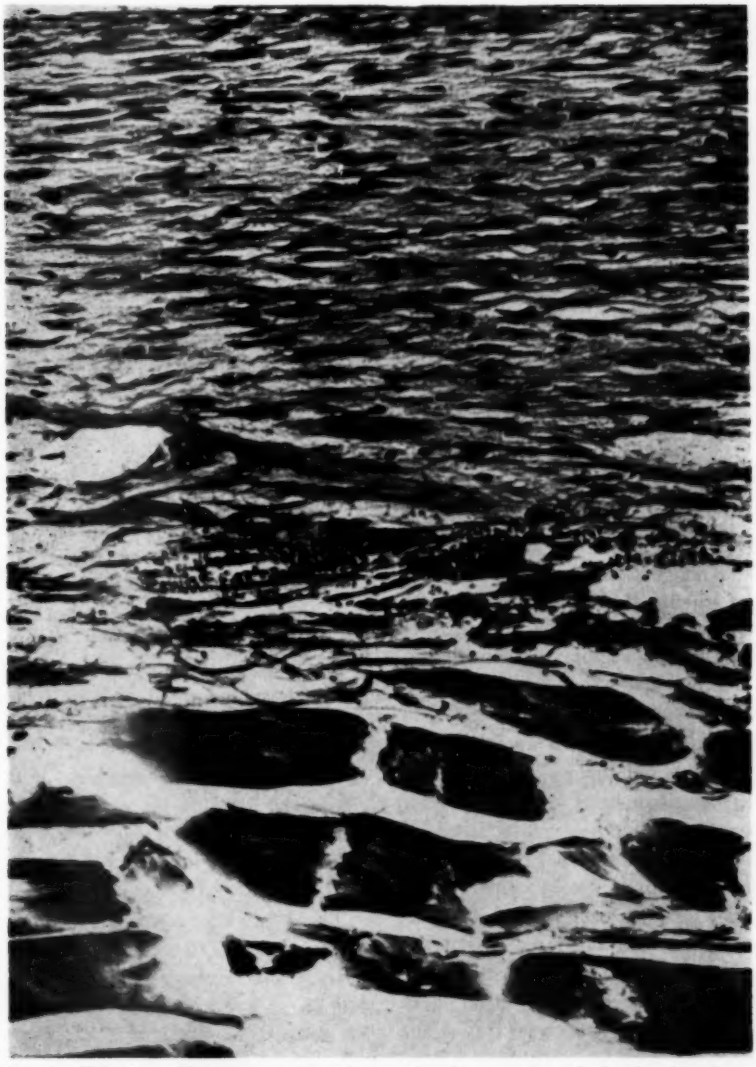


Fig. 4.—This is a high-power photomicrograph of a portion of the irradiated region, shown in Figure 2. The empty space above the tissue represents the bursal space. A poorly vascularized dense pannus has grown over the fascia. Beneath the fascia there are small vascular channels which have originated from the host. There is very little resorption of muscle of the graft. The musculofascial zone is narrow. Stromal penetration by cells of the host is minimal despite the availability of a blood supply. Proliferation of cells of the graft is equally inconspicuous. Admixture of cells of the graft and the host is negligible. The inflammatory reaction, so outstanding in the unirradiated half of the graft, is absent (see Figure 3).

tions by which one-half of the graft was shielded from effects of radiation with a lead plate. By this means all variables other than radiation were eliminated (Figs. 1 and 2).

Comment

Microscopic analysis of local tissue interactions between musculofascial grafts and the host in a sterile intramuscular environment has led us to study factors responsible for failure of homografts to survive. Autologous grafts showed orderly sequences of degeneration, absorption, and organization. Homologous grafts showed similar sequences interrupted by inflammatory reactions which interfered with reparative collagen deposition and fibroblastic proliferation. The region directly beneath the fascia of each graft was of greatest interest in determining whether the transplant was an autograft or homograft. The stimulus responsible for the inflammatory aspects of the host-homograft incompatibility reaction seemed to be concentrated or trapped in this region, and so characteristic interactions were intensified and more easily recognized here.

Previous studies in which musculofascial grafts were exposed to low and high temperatures in vitro prior to implantation revealed a change in host-graft tissue interactions when the exposure was above and below certain critical temperatures.[1] This change was characterized by failure of penetration of vascularized stroma of the host into the graft, and so collagenous encapsulation with delayed modification of architecture of the graft was the result. Apparently, the host was unable to distinguish between its own tissue and similar homologous tissue as grafts. Also, tissues of the grafts reacted equally to the host.

The present studies have shown that 90,000 to 10,000 r of x-irradiation given to grafts in vitro prior to implantation produced changes leading to an inert host-graft tissue interaction similar to the one obtained by exposure of grafts to low and high temperatures in vitro before implantation (Table).

When lower doses, 5000 to 1000 r, were used, the stimulus to vascularization of grafts was preserved but formation of granulation tissue in the grafts was negligible and homografts did not excite an inflammatory reaction. It was apparent that the stimulus to vascularization was not necessarily related to proliferative activities of grafted tissue or excitation of classical host-graft interactions (Table).

The doses of 750 r and 500 r appeared to be in the neighborhood of a lethal dose for at least some cells of the graft. This is in agreement with effects of x-irradiation upon mouse fibroblasts in tissue culture.[4] Though after a dose of 1000 r vascularization of the graft occurred, the inflammatory aspects of the homologous reaction became recognizable only after a dose of 750 r or less. Proliferative admixture of cells from the host and graft was therefore regarded as an important prerequisite for stimulation of inflammation. The proliferative admixture of cells of the host and graft became increasingly conspicuous after doses of less than 750 r. Cells from both sources contributed to the development of new structure in the musculofascial zone of the graft. At the same time, distinctive host-homograft reactions occurred in this location but only after the least irradiation (100 r and 250 r) (Table).

These studies have shown that when musculofascial tissue was irradiated in vitro before implantation, its rate of deterioration as a graft was reduced. This was in contrast to the effects of x-irradiation of skeletal muscle in vivo where severe histological alterations have been described after treatment with 50,000 r.[5] Also, x-radiation of musculofascial tissue in vitro at low doses as described produced no increased or abnormal proliferation of cells of the graft after subsequent implantation. Retardation of proliferative activity was the rule. Furthermore, neither high nor low doses of x-irradiation in vitro be-

fore implantation increased the capacity of autograft or homograft to elicit an inflammatory reaction on the part of the host. It was also noted that pathologic calcification of muscle which often follows loss of viability of muscle cells was no more conspicuous in irradiated than in control grafts.

Finally, the data indicate that an understanding of the pathogenesis of undesirable features of host-homograft reactions is closely linked to an understanding of the mechanisms responsible for desirable features of host-autograft reactions. The desirable features of the one reaction were lost under about the same conditions of radiation as loss of undesirable features of the other reaction. The stimulus to the excitation of both reactions seems bound up in an analysis of what constitutes cell "viability." In getting at this problem, it may be useful to take a critical look at concepts of cell "viability" with the view of determining whether there are facets or levels of "viability" which call forth a spectrum of tissue interactions. If these exist, further study may best be directed toward an attempt to disclose the particular level or facet of cell "viability" required to induce each of the four forms of observed host-graft interactions: the first being classical interactions (0 to 250 r), the second being indeterminate feeble reactions (500 to 750 r), the third being only stromal penetration and vascularization of the graft (1000 to 5000 r), and the fourth being an inert interaction with only fibrous encapsulation of the graft (10,000 to 90,000 r).

Summary

After resection from the donor and prior to implantation in the recipient, autologous and homologous musculofascial tissue from rabbits was irradiated in vitro with x-rays. A microscopic study of the grafts, one to three weeks after implantation, led to the following observations. Irradiation with 100 to 250 r did not interfere with development of classical host-graft interactions. Irradiation with 500 r and 750 r reduced the intensity of host-autograft reactions and eliminated the capacity of homologous tissues to induce distinctive host-homograft reactions. Irradiation with 1000 to 5000 r reduced all grafts to the common state where the host-graft interactions were identical. These were characterized by vascularized stromal encapsulation and penetration of the graft by tissues of the host without much response by tissues of the graft. Irradiation with 10,000 to 90,000 r led to a common inert interaction between all grafts and the host, so that the grafts tended to persist unchanged, being encapsulated by fibrous tissue of the host without vascularized stromal penetration or other significant admixture of tissues of the host with those of the graft. These data indicated that advantage may be taken of the apparent levels of increasing inactivation of properties of grafts to assist in a study of factors responsible for desirable and undesirable interactions between tissues of the host and tissues of unmodified grafts.

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The Effects of Prolonged Hypothermia on the Rat

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Although extensive physiologic studies have been performed relating to the effects of hypothermia in various species of animals,[1-4] the histologic and histochemical changes have not received much attention. Histologic and histochemical studies on the liver, heart, kidneys, and adrenals of the rat following prolonged hypothermia are reported here.

Material and Methods

Male albino rats of the Sprague-Dawley strain (weighing 230 to 280 gm. each) were used. The animals were made hypothermic, with use of light ether anesthesia, by surrounding them with plastic bags filled with cracked ice. When the rectal temperature reached 22 C (71.6 F) the ice bags were removed; 2.8 mg. of pentobarbital (Nembutal) sodium per 100 gm. of body weight was given intraperitoneally to control shivering, and the animals then were placed in an environment at 15 to 20 C (59 to 68 F) to maintain the body temperature between 18 and 20 C (64 and 68 F). When shivering became apparent, additional pentobarbital sodium or ether was given. If the temperature dropped below 18.5 C (65 F) the animal was removed to a warmer environment until the body temperature rose to 20 C.

The body temperatures were obtained with use of all-metal dial-type thermometers. These were inserted in the rectum and left in place for the duration of the experiment. Temperatures were observed continuously while the animals were undergoing the initial induction of hypothermia. After the temperature had been stabilized, readings were taken at 15- to 20-minute intervals.

Groups of four animals each were observed after 2, 4, 6, 8, 12, and 18 to 23 hours of hypothermia. Fasting controls represented those animals deprived of food for corresponding periods but not kept hypothermic. Normal controls consisted of animals of the same stock and weight not subjected to hypothermia. Tissue from the heart, liver, kidney, and adrenal glands was taken for histologic and histochemical study. In addition to hematoxylin-and-eosin stains, succinic dehydrogenase activity was determined according to the method of Seligman and Rutenberg.[6] Cytochrome oxidase activity was demonstrated with use of the method of Gomori[7] and the Nadi reaction. The periodic acid-Schiff reaction was used to determine the glycogen content of the heart, liver, and kidneys after fixation in Bouin's solution for 18 to 20 hours at 4 C. Identical diastase-digested sections served as controls. The maximum amount of glycogen observed in a tissue was graded 4+. Alkaline-phosphatase activity was demonstrated by the Ca-Co method.[8] Formalin-fixed tissue was studied for fat with Sudan IV and oil red O.

Results

Heart.—No morphologic changes were noted in the hypothermic animals when hematoxylin-and-eosin-stained sections were examined. There was no alteration in succinic dehydrogenase and cytochrome oxidase activity. Sudanophilic substances were not seen in the myocardium of hypothermic animals or in the controls.

Alkaline-phosphatase studies on fresh-frozen material outlined myocardial capillaries but showed no reaction in myocardial fibers. The distribution and amount of alkaline phosphatase was identical to that seen in controls.

As shown in the Table, the myocardial glycogen of normal rats and of those exposed to reduced temperatures up to four hours was graded as 1+ (Fig. 1). At six hours of hypothermia there was a perceptible increase in glycogen content, the histologic grading being 2+. This increase

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Average Myocardial and Hepatic Glycogen in Hypothermic and Fasting Rats

	Hours of Hypothermia							
	0	2	4	6	8	12	16	18–23
Heart								
Hypothermic	1+	1+	1+	2+	2+	3+	3+	4+
Fasting	1+	1+	1+	1+	1+	2+	2+	4+
Liver								
Hypothermic	4+	4+	4+	4+	3+	2+	2+	±
Fasting	4+	4+	4+	4+	4+	4+ *	2+	2+

* One of four rats showed a 2+ reaction. The remaining three rats showed 4+ glycogen.

was more pronounced at 12 hours (3+) and was evident in greater quantity at 18 to 23 hours (4+) (Fig. 2). In the fasting controls, the myocardial glycogen was 1+ for the first 8 hours, was 2+ in 12 hours, and finally rose to the maximum at 24 hours.

Liver.—There were no morphologic changes or alterations in succinic dehydrogenase, cytochrome oxidase, or alkaline phosphatase activity in the liver. The amount and location of demonstrable sudanophilic substance was not changed by hypothermia.

Glycogen was seen in abundance in normal controls and persisted in similar quantities through six hours of hypothermia (Fig. 3). In eight hours (Table) the liver glycogen diminished to 3+ and in 12 hours there was a marked decrease in glycogen (2+) and that which remained was for the most part located in the central zones. In 18 to 23 hours of hypothermia, there was almost a complete disappearance of glycogen as demonstrated histochemically. The small amount that remained was seen in a narrow zone of cells around some of the central veins and in an occasional cell throughout the liver (Fig. 4). When normothermic animals were fasted 2, 4, 6, and 8 hours, no loss of liver glycogen was demonstrated. After 12 hours of fasting, one rat in a group of four showed a diminution of liver glycogen. At 16 and 24 hours of fasting, there was a 2+ glycogen content in the livers of all the rats.

Kidney.—Hematoxylin-and-eosin-stained preparations revealed no morphologic changes. Cytochrome oxidase, succinic dehydrogenase, and alkaline-phosphatase activity was unaltered by hypothermia. No fat or glycogen was seen in the controls or hypothermic animals.

Adrenals.—No evidence of glycogen was seen, although the zona reticularis and the medulla contained small traces of periodic

Fig. 1.—Rat myocardium, four hours of hypothermy. Small amount of glycogen is deposited diffusely. Periodic acid-Schiff and diastase-digestion technique; × 360.

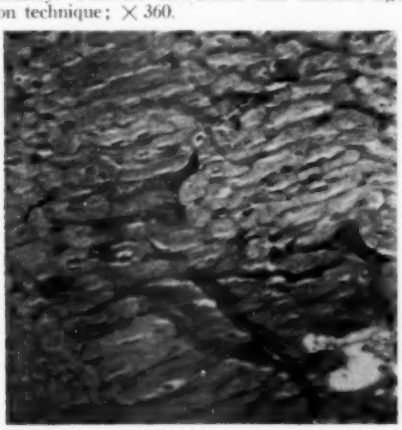
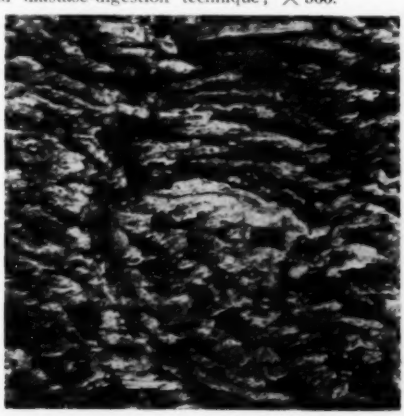


Fig. 2.—Rat myocardium, 20 hours of hypothermy. Glycogen more abundant and more granular than in Figure 1. Periodic acid-Schiff and diastase-digestion technique; × 360.



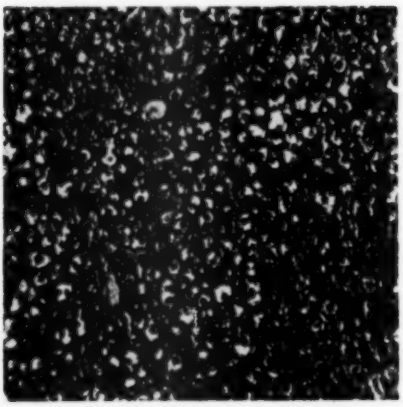


Fig. 3.—Rat liver, six hours of hypothermy. Glycogen is abundantly deposited in all cells similar to that seen in controls. Periodic acid-Schiff and diastase-digestion technique; × 360.

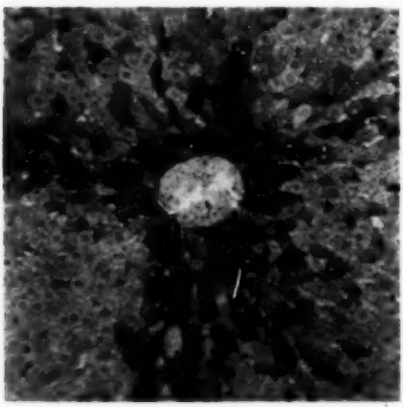


Fig. 4.—Rat liver, 20 hours of hypothermy. Only a few cells contain glycogen, and these are in the area of the central vein. Periodic acid-Schiff and diastase-digestion technique; × 360.

acid-Schiff-positive material in the control and the hypothermic rats. An abundance of sudanophilic material was present in the zona fasciculata; smaller amounts were present in the zona glomerulosa and zona reticularis, and none, in the medulla. This deposition was not changed by hypothermia. Alkaline phosphatase was absent in control and hypothermic rats.

Comment

In this study it was shown that during prolonged hypothermia in the rat there was no reduction of succinic dehydrogenase, cytochrome oxidase, or alkaline phosphatase as demonstrated histochemically in the heart, liver, and kidney. This would indicate that during this period neither were the enzymes destroyed nor was their production prevented. This does not mean that there was no reduction of activity during the period of hypothermia.

There was no increase of fat in the adrenals or fatty changes in the liver, heart, and kidney. These findings in the rat differ from those reported in the dog by Knocker,[5] who found that two and one-half hours of hypothermia resulted in a marked increase of lipid in the zona reticularis of the adrenals and in the distal convoluted tubules of the kidneys as well as fatty changes in the liver.

In the dog, blood sinusoids were dilated and congested and adjacent liver cells were void of demonstrable glycogen. In the rat there was no change in the sinusoids and the disappearance of glycogen occurred first in the peripheral areas of the lobules and later in the cells adjacent to the central veins.

The different results might possibly be explained on the basis of species difference, since it is known that the rat reacts physiologically more favorably toward prolonged hypothermia than the dog.[8]

Hypothermy affects the quantity of myocardial and liver glycogen. In the hypothermic rat there was a definite increase in the amount of myocardial glycogen, whereas liver glycogen decreased. This effect is more than a factor of starvation over the extended period of hypothermy, since the glycogen shift occurs much earlier than in fasting animals (Table).

On the basis of activity requiring a greater utilization of glycogen, one would expect the depletion of liver glycogen to be more rapid in the more active fasting group than in the hypothermic group. Since this is not true, hypothermia must affect the glycogen shift.

Summary

Histologic and histochemical observations were made of the heart, liver, kidneys, and adrenal glands of rats during hypothermia. It was found that no significant alterations occur in succinic dehydrogenase, cytochrome oxidase, and alkaline phosphatase activity in the heart, liver, and kidneys. Contrary to evidence obtained in the dog by others, no alteration was noted in the fat content of the adrenals, kidneys, and liver. During hypothermia there was a loss of liver glycogen and an increase in myocardial glycogen.

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Case of Wegener's Granulomatosis

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Introduction

Wegener was the first to define a clinical and pathological entity that is called now Wegener's granulomatosis.[1] Even before Wegener, a case was described by Klinger as a variant of polyarteritis nodosa which—it is felt now—may represent the first published case of Wegener's granulomatosis.[2]

New interest in this subject was aroused by the recent review of Fahey and co-workers.[3] These authors were able to collect only 22 cases of this entity from the literature, and they added 7 of their own. As stated by Fahey, Wegener's granulomatosis is characterized by three features: (*a*) necrotizing granulomatous lesion in the upper airways or lower respiratory tract or both, (*b*) generalized focal vasculitis, (*c*) necrotizing glomerulitis. Since this review, other cases have been published. Plumer and co-workers collected 35 cases from the literature and added two of their own.[4] The small number of reported cases alone justifies the addition of another case to the literature. The predominance of the granulomatous process and the relative absence of vasculitis in our patient is also noteworthy.

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Clinical History

A 26-year-old white man was admitted to the Westside Veterans' Administration Hospital, Chicago, on Sept. 24, 1954, because of fever, cough, and weight loss of approximately one month's duration. He was in good health six months prior to admission except for two brief episodes of fever and cough. The first febrile period was treated with an unknown oral medication, and the second febrile period was treated with penicillin. Clinical improvement occurred on both occasions, except for the persistence of a slight cough. One month prior to admission his cough increased in severity; fever was again experienced, and anorexia became so marked that a 20 lb. weight loss ensued. Cough was productive of only a small amount of white sputum, which subsequently became blood streaked. Temperature elevations up to 102 F, accompanied by profuse sweating, were experienced daily.

Other than exposure to tuberculosis in 1948, his past history was noncontributory. There were no known allergic states and no known drug sensitivity. Prior to admission he had received a brief course of chlortetracycline (Aureomycin), with no apparent effect upon his symptoms.

Physical Examination.—The patient was acutely ill, febrile, and in moderate respiratory distress. The blood pressure was 124/84 mm. of mercury; respirations, 20 per minute, and pulse rate, 92 per minute. The nasal mucosa was inflamed and edematous. Examination of the heart, lungs, and abdomen revealed no abnormalities. Lymphadenopathy was not present, and neurological examination was within normal limits.

Laboratory and Special Examinations.—The roentgenogram of the chest (Fig. 1) showed a noncalcified right hilar mass. Subsequent roentgenograms showed a rapid enlargement of this mass, with development of a right pleural effusion. The initial laboratory data revealed a hemoglobin of 14 gm. per 100 cc.; red blood cell count, 4,500,000 per cubic millimeter, and white blood cell count, 13,000 per cubic millimeter, with 8% stab forms, 75% neutrophils, 11% lymphocytes, 2% monocytes, and 4% eosinophils. The cardiolipin

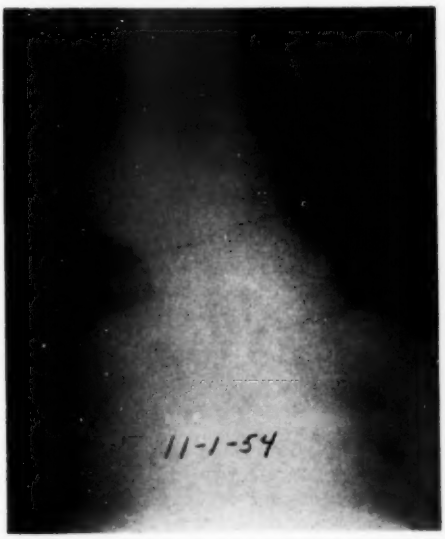

Fig. 1.—Roentgenogram of chest, demonstrating large right hilar mass.

microflocculation test for syphilis, heterophile antibody examination for infectious mononucleosis, and polyagglutination tests for routine bacterial agents were negative. Both aerobic and anaerobic blood cultures were negative. Sputum cultures showed the presence of α-streptococci and Neisseria catarrhalis but were negative for acid-fast bacilli and fungi. The total serum protein was 7.7 gm.; albumin, 4.9 gm., and globulin, 2.9 gm. per 100 cc. Serum alkaline phosphatase (Bodansky method) was 3.3 units per 100 cc. and thymol turbidity, 1.2 units, while the cephalin flocculation test showed a trace reaction in 24 hours and a 1+ reaction in 48 hours. The sulfobromophthalein test revealed 23% retention at 45 minutes. The total van den Bergh reaction for bilirubin was 0.3 mg. %, and the indirect van den Bergh reaction, 0.2 mg. %. The non-protein nitrogen was 22 mg. per 100 cc. The urine had a specific gravity of 1019; tests for albumin and sugar were negative. Microscopic examination of the urine sediment showed a rare red blood cell and an occasional white blood cell per high-power field.

Course in Hospital.—The patient had a continuous fever, with oral temperatures ranging from 99.6 F to 104 F. Peak temperatures occurred in the early evening hours and were soon followed by a profuse drenching sweat. His cough increased in severity, and moderate right sided pleuritic pain was experienced. Tuberculin skin tests were negative, while the histoplasmin skin test showed a 3+ reaction at 48 hours. A bone marrow examination on Sept. 30, 1954, demonstrated an eosinophilia of 8%. Bronchoscopy was performed soon after admission, and no abnormality was found. Bronchial washings were negative for tumor cells. A liver biopsy was done on Oct. 5, 1954, and showed the presence of focal hepatitis. The febrile state of the patient was unaffected by courses of penicillin and streptomycin.

In view of the rapidly expanding hilar mass and the lack of positive diagnostic information, the patient was transferred to the surgical service for an exploratory thoracotomy. At this point it was the clinical impression that the patient had either a lymphoma or a bronchogenic carcinoma. On exploration of the right chest a smooth rubbery mass measuring 15 cm. in diameter was found, involving the right upper lobe. The mass extended across the small fissure and was adherent to the pericardium overlying the right auricle and a portion of the ascending arch of the aorta. The mass was also adherent to the anterior chest wall. Adequate biopsy material was obtained; portions of tissue were cultured for tuberculosis, fungi, and predominating bacterial organisms. These later studies proved negative. Microscopic examination of the biopsy tissue demonstrated a necrotizing inflammatory granuloma with associated acute and chronic pneumonia. After surgery the febrile state continued; leukocytosis with increasing eosinophilia and anemia developed. For the first time painful necrotic ulcers on the lateral margins of the tongue were observed. A biopsy specimen of one of these lesions showed nonspecific necrotizing granulomatous inflammation. This granulomatous process had a distinct resemblance to the lung lesions found on biopsy. In addition to the tongue lesions, other changes in the patient's physical condition were noted. The liver became enlarged, and bilateral peripheral edema became apparent. Pain, redness, and swelling of several metacarpal joints developed.

Considering that a disseminated fungus infection was a strong clinical possibility, the patient was given nystatin (Mycostatin) and then subsequently 2-hydroxy-stilbalimidine. These drugs caused no improvement. During the fifth week of hospitalization, his condition began to deteriorate rapidly. Cough and hemoptysis became more marked. Hoarseness lead to aphonia. Furuncle-like lesions developed around the elbows and the extensor surface of both forearms. These lesions had necrotic centers which were covered by a black crust. Marked conjunctivitis of his left eye was noted. During this period, laboratory changes likewise became apparent. The sulfobromophthalein test showed 16% retention, while the serum alkaline phosphatase rose to 12.1 units. The total serum protein became 6.4 gm. per 100 cc., with albumin of 3.7 gm. per 100 cc. and globulin of 2.7 gm. per 100 cc. Thymol turbidity test showed 0.6 units.

The white blood cell count was 28,100 per cubic millimeter, with 83% neutrophils, 7% lymphocytes, 2% monocytes, and 8% eosinophils. The day prior to death, the nonprotein nitrogen was 56 mg. per 100 cc. The urine examination showed a specific gravity of 1020 and a 2+ reaction for albumin. Many coarse and fine granular casts, with 4 red blood cells and 8 white blood cells per high-power field, were found on microscopic examination. On the day of death, his respiratory rate rose to 50 per minute, a supraventricular tachycardia with a rate of 160 per minute developed, and the rectal temperature reached 106 F. The patient became comatose and died on Nov. 3, 1954.

Autopsy Report

Gross

Mouth: Many small ulcers of the tongue were seen.

Larynx and Trachea: The mucosa showed many grayish-white punctate ulcers.

Chest: A recent well-healing thoracotomy wound was seen in the right fifth intercostal space. The right pleural cavity was obliterated by dense fibrous adhesions; 150 cc. of slightly cloudy yellow fluid was found in the left pleural cavity. Combined weight of the lungs was 2520 gm. The right upper lobe of the lungs was globular in shape, and its cut surface revealed a yellow spherically shaped mass that suggested extensive necrosis and occupied almost the entire lobe. Only a rim of apparently normal lung tissue was noted peripherally. The remaining right lung showed no evidence of consolidation. The left lung showed hyperemia. Upon palpation a spherically shaped mass was felt in the left upper lobe. Its cut surface resembled the mass found in the right upper lobe, although it was smaller. It measured 3×4 cm. in diameter. The bronchi were lined by a granular and thickened mucosa that revealed punctate areas of superficial erosion.

Liver: The liver was markedly enlarged and weighed 2860 gm. The cut surface showed preserved architectural pattern with hyperemia.

Spleen: The spleen was large; it weighed 710 gm. and measured 20×14×6 cm. The organ was attached to the surrounding tissues by dense fibrous adhesions. In the process of removal, the splenic capsule tore freely and the organ appeared to be denuded. The cut surface of the organ was grayish pink in color, resembling raw meat. Numerous small cystic spaces were honey-combing the tissue. Examination of the splenic vessels showed no evidence of thrombosis.

Kidneys: Each of the kidneys was enlarged. The right kidney weighed 250 gm., and the left kidney weighed 260 gm. The capsules stripped with some difficulty. There were no petechiae. The cut surface showed a thickened cortex with preserved striations.

Gastrointestinal Tract: The esophagus showed many areas of superficial ulceration, mainly in the lower third. The stomach was not remarkable.

The small intestine showed many mucosal ulcers. The latter were oval in shape and measured up to 3 cm. in diameter. Their longitudinal diameter was parallel to the longitudinal axis of the bowel. The large intestines contained tar-like fecal material.

Microscopic

Heart: Sections of the heart showed a number of foci of paravascular fibrinoid

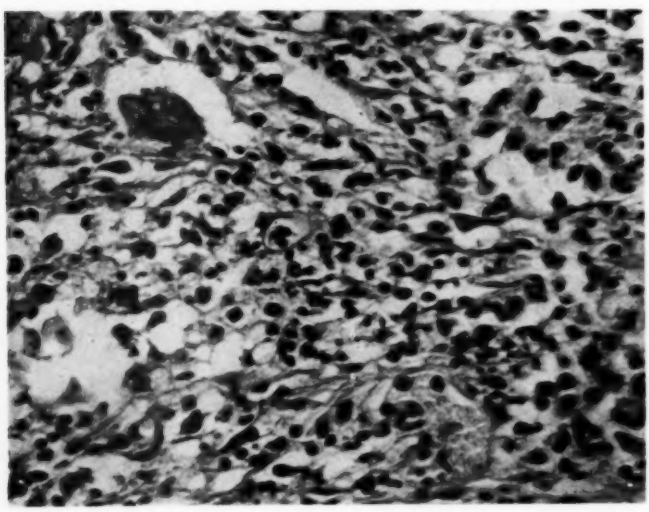

Fig. 2. — Microscopic section of pulmonary mass showing granulomatous inflammation with giant cell.

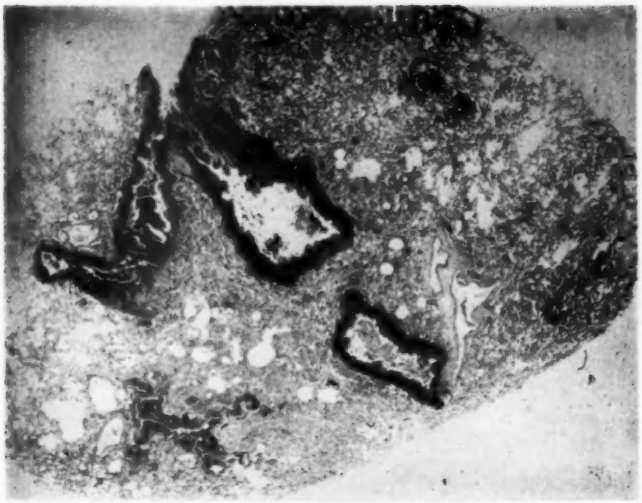

Fig. 3.—Diffuse bronchial involvement.

degeneration. Adjacent to these foci were small aggregates of lymphocytes, plasma cells, and some large mononuclear cells. No Aschoff cells were noted.

Lung: Sections showed large areas of necrosis, with marked proliferation of mononuclear cells that resembled epithelioid cells. Occasional multinucleated giant cells were encountered. Some were of the appearance of Langhans giant cells; others resembled foreign-body type of giant cells (Fig. 2). Some of the proliferating peripheral cells had a foamy cytoplasm. Fat stain was positive in these areas. Sections of the grossly uninvolved lung showed extensive mucosal ulceration of practically all the bronchi, with dilatation of the bronchial lumina (Fig. 3).

Liver: Sections showed numerous small granulomatous lesions. They were composed of polyhedric epithelioid cells that showed a somewhat polymorphic vesicular nucleus (Figs. 4 and 5). Reticulum stain showed a well-developed reticular network between these cells (Fig. 6). A few of the granulomata showed early necrosis. An occasional giant cell was also noted. In a

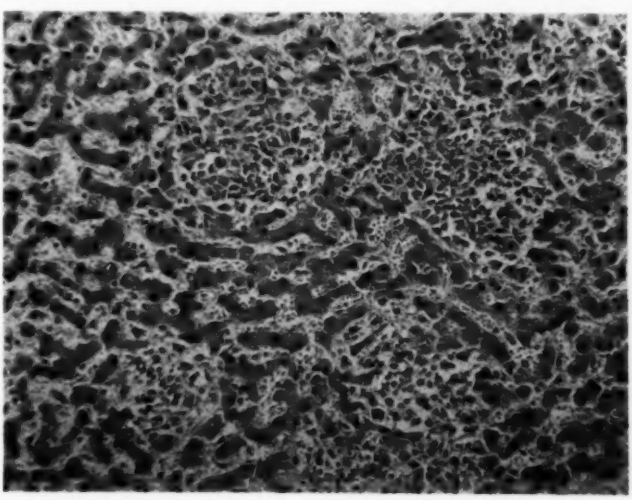

Fig. 4. — Microscopic section of liver with multiple granulomata.

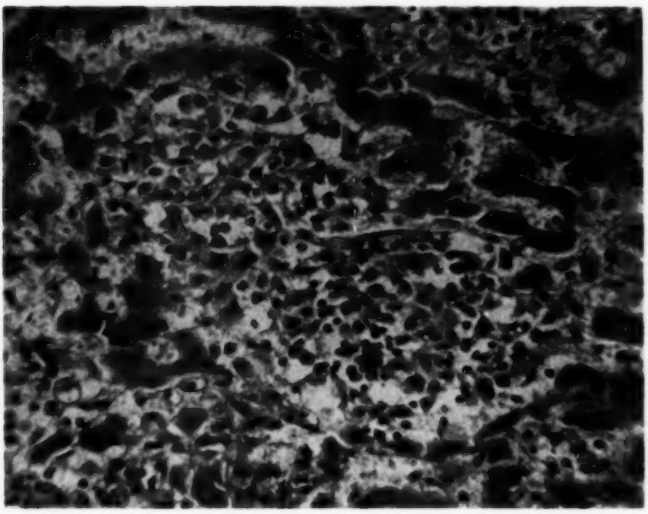

Fig. 5.—Close-up of granulomata in liver.

few areas there was suggestion of small venous involvement, with granulomatous infiltration of the wall and fibrinoid necrosis.

Spleen: Sections showed fibrosis of the capsule, with attached omental fat. There was diffuse and also focal proliferation of epithelioid-like cells, with an occasional multinucleated giant cell. The bulk of the splenic sections showed complete and also incomplete necrosis. In places Malpighian bodies could be made out, with necrotizing involvement of the central arteries. The splenic necrosis was caseous in character, with areas of liquefaction and pseudocyst formation. Section of the splenic organ in the hilar region showed a medium-sized artery occluded by a recent thrombus. The arterial wall was of normal appearance.

Kidneys: Sections showed widespread healing and also acute necrotizing focal glomerulonephritis (Fig. 7). About 80% of the glomeruli showed the disease process.

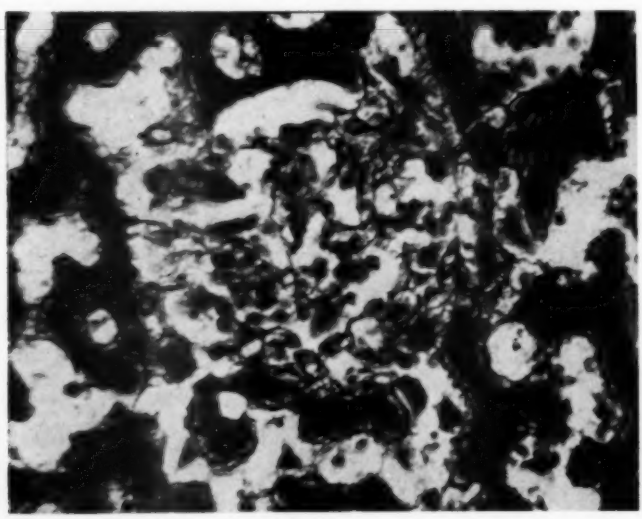

Fig. 6.—Reticulum stain of liver granuloma.

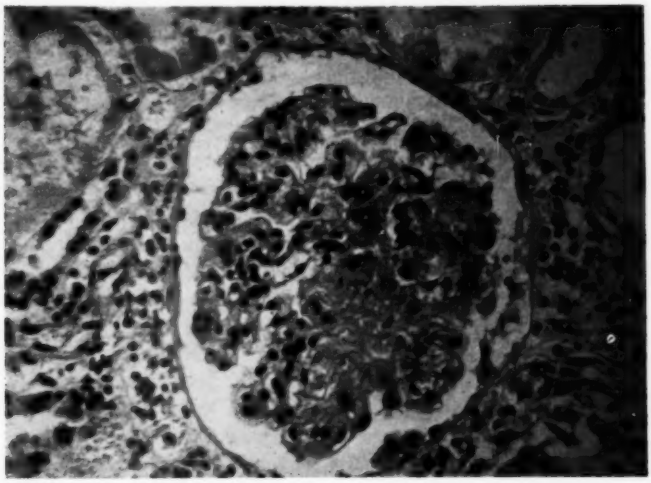

Fig. 7. — Microscopic section of kidney, revealing focal necrotizing glomerulonephritis.

There was no involvement of the small and medium-sized blood vessels. Periodic acid-Schiff (PAS) stain showed considerable thickening of the basement membrane of loops in focal areas of the glomeruli. Heidenhain's aniline blue stain showed most of the glomerular lesions to be fibrous in character.

Lymph Nodes: Sections of various lymph nodes showed marked diffuse reticulum hyperplasia with extensive areas of necrosis. One of the sections showed a single venous vessel within the uninvolved lymphoid tissue with fibrinoid necrosis of part of the vessel wall.

Special Stains: Sections from lung, liver, spleen, and lymph nodes were stained for acid-fast bacilli (modified Ziehl-Neelsen) and fungi (PAS and Gomori's methenamine silver nitrate). These stains proved to be negative.

The clinical picture of Wegener's granulomatosis has been described by Fahey.[3] Rhinitis or sinusitis may give the first symptoms, or attention may first be focused on the lung because of cough, hemoptysis, or evidence of nonresolving pneumonia. X-ray examination of the chest may reveal a nonspecific pulmonary infiltration or nodular densities, which suggest neoplasm or tuberculosis. Johnson's patient with multiple pulmonary lesions was suspected of having metastatic carcinoma.[5] The first case of Postel and Laas was thought to have a lung tumor, and the second case of the same authors was diagnosed as tuberculosis with kidney involvement.[6]

The course of our patient's disease showed many features of previously documented cases. Fever was almost continuous and unaffected by a variety of antibiotics. The pulmonary lesion was characterized by rapid expansion accompanied by chest pain and hemoptysis. Only in the latter part of his hospital stay was the general systemic nature of the disease apparent. Liver involvement, as noted in this patient, has not often been recorded in other cases. Plumer et al., noted liver enlargement in their second patient.[4] In our case, the alkaline phosphatase rose from 3.3 units (Bodansky) to 12.1 units in the latter stages of the disease. Eosinophilia has been variable in several reported cases. Bone marrow and peripheral blood smears showed definite increase of the eosinophils in our case. Weinberg described tongue ulcers in this syndrome.[8] The extreme pain of these ulcers in our patient is worthy of comment. Although the kidneys were directly responsible for the fatal outcome in 86% of cases, this did not seem to be the mechanism in our patient.

His preterminal nonprotein nitrogen was not markedly elevated.

The most notable findings of the autopsy were (*a*) necrotizing granulomatous process in lung and spleen, (*b*) diffuse ulcerative and necrotizing bronchitis, (*c*) small granulomatous lesions diffusely infiltrating the liver, (*d*) focal necrotizing glomerulonephritis, (*e*) focal vasculitis (rare).

The necrotizing process in the lung and spleen has originally been considered to be an infarction, secondary to angiitic changes. Klinger, who was the first to publish a case of this entity, was already impressed by the atypical histological features of the "infarct."[2] These lesions did not show the usual picture of bland infarcts. The necrotic areas were penetrated by leukocytes and histocytes. Postel and Laas described changes of the spleen as infarcts but were unable to demonstrate a cause in the vessels.[6] Godman and Churg thought that the respiratory granulomata represented the combined result of a primary affection of the respiratory tissues and subsequent or concomitant regional angiitis.[7] We noted in our case that the blood vessels as they entered the granulomatous areas were free of pathology. Only within the necrotizing granulomatous lesions were thromboangiitic changes present. It is, therefore, our impression that the necrotizing granulomatous process in Wegener's granulomatosis is a primary lesion within the affected organ, independent in its evolution from an angiitic process. No generalized vasculitis was found.

Fahey and co-workers stated that the generalized focal vasculitis was an indispensable part of the Wegener's triad, namely, granuloma, vasculitis, and glomerulitis.[3] Godman and Churg, who described the pathological findings in the Fahey series, emphasized that in some cases the vascular lesions were prominent and the outstanding feature of the disease, while in others the vascular lesions were very few and the granulomatous features dominated the scene.[7] Except for rare instances of focal necrotizing angiitis in the liver and in an occasional area of a lymph node, angiitic changes were only noted within the granulomatous process, particularly in the spleen of our patient.

Plumer and co-workers stated that the histology of their cases resembled the microscopic form of polyarteritis nodosa but that it differed from it by the presence of the necrotizing granulomata, mostly in the respiratory tract.[4] These author considered Wegener's granulomatosis a subgroup of polyarteritis nodosa. Rose and Spencer reviewed 111 cases of polyarteritis nodosa and divided them into two groups, those with and those without lung involvement. Thirty-two patients showed polyarteritis with lung involvement, twenty-seven (83%) of these cases showed necrotizing lesions other than infarcts. These authors apparently did not accept Wegener's granulomatosis as a disease entity separate from the group of polyarteritis nodosa.

The widespread disease of the bronchi was remarkable. Practically all bronchi showed ulceration of the mucosa with dilatation of the lumina. Fienberg previously had noted cavities within the granulomatous process and conjectured that they were due to dilatation of the small bronchi.[10] One of the patients reported by Sweeney and Baggenstoss similarly showed numerous cavities, which were thought to represent bronchiectases.[11]

Liver involvement as noted in this patient had not been frequently mentioned in other patients with Wegener's granulomatosis. The granulomatous process in the liver was extensive, with numerous small granulomata spread diffusely throughout the liver. The granulomata were composed of epithelioid-like cells, similar to the ones seen in tuberculosis, although the vesicular nuclei were rather pleomorphic in appearance. A beautiful reticular fibrillar network was seen between these cells. Focal necrosis was noted within the granulomata, and this necrosis may support the supposition that the necrosis in the larger granulomata of

the lung and spleen was part of the granulomatous process and not vascular in origin. The cells of the reticuloendothelial system in granulomatous inflammation undergo many successive morphologic changes. These are proliferation, degeneration, death, and dissolution.[12] Plumer et al.[4] also reported granulomata of the liver in one of their patients.

Despite the absence of significant NPN elevation, the kidney sections of our patient showed the picture of extensive focal necrotizing glomerulonephritis as it has been consistently reported by other authors. The resemblance of these glomerular lesions to the ones seen in subacute bacterial endocarditis and in the microscopic form of periarteritis nodosa has been already noted by Godman and Churg.

Summary

A case of Wegener's granulomatosis is presented. The necrotizing process in the lung and spleen, the focal necrotizing glomerulonephritis, the granulomatous involvement of the liver, and the presence of tongue ulcers are described. The predominance of the granulomatous process over the vascular inflammatory changes is emphasized.

The medical illustration department of the West Side Veterans' Hospital prepared the illustrations for this study.

Veterans' Administration Westside Hospital, 2030 W. Taylor St. (12).

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Potassium Deficiency and Alkalosis in the Rat

Absence of Histopathologic Changes in the Brain

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The effects of potassium deficiency and alkalosis on the kidney and myocardium of the rat have been well studied.[3,4,7,8] This experiment was undertaken to investigate the histopathologic changes in the brain which might occur in this situation.

Methods

Seventeen Wistar strain white male rats weighing approximately 240 gm. were randomly divided into a control group of eight and a test group of nine animals. They were rendered potassium deficient and alkalotic by a modification of a dietary method used by Cooke and his associates.[1,2] The basic diet consisted of dextrin, 500 gm.; hydrogenated fat, 150 gm.; casein, 200 gm.; corn oil, 200 gm.; a vitamin mixture,[6] 1.185 gm.; cod liver oil, 4 ml., and alpha tocopherol, 40 mg. Forty grams of the Hawk-Oser salt mixture[5] was added to the control diet. Twenty-three grams of this mixture with the exception of sodium and potassium salts was added to the test diet. The drinking solution for the control animals contained sodium, 150 mEq. per liter; bicarbonate, 150 mEq.; potassium, 10 mEq., and chloride, 10 mEq. The drinking solution for the test animals contained sodium, 154 mEq. per liter; bicarbonate, 150 mEq.; potassium, 1 mEq., and chloride, 5 mEq. Both groups were allowed to eat and drink ad libitum.

After 10 weeks, the animals were killed by cardiac puncture and exsanguination with use of light ether anesthesia. Serum carbon-dioxide-combining power and potassium were measured, but through a laboratory error only two determinations of potassium were obtained. The brains were divided into two hemispheres; one was sectioned in the frontal plane, and the other, in the sagittal plane. The sections were studied with Weigert, toluidine blue, and hematoxylin-and-eosin stains. The brains of two controls and two test animals were studied with an astrocyte gold stain.

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Results

At the onset of the experiment, the weights of the control and test rats were similar. The mean weight and standard deviation of the control animals were 236 ±18 gm. and that of the test animals, 242 ±16 gm. However, at the end of the experiment, 10 weeks later, the control rats had a mean weight and standard deviation of 365 ±50 gm., while the mean weight and standard deviation were only 271 ±25 gm. in the test rats. In the control group, the mean carbon dioxide in five animals was 20 mEq. per liter, in contrast to 33 mEq. per liter in eight test animals. The serum potassium was 5.6 mEq. in a control rat and 3.4 mEq. per liter in a test rat.

The test group was less active than the control, but neither twitching nor paralysis was observed. The fur of these animals lost its sheen and shed in many places.

There were no changes in the sections of brain in either group studied with the Weigert, toluidine blue, hematoxylin-and-eosin, and astrocyte gold stains.

Comment

The serum levels of carbon dioxide and potassium in the control and test rats compare well with those reported by Cooke and his associates.[1,2] These authors carried out their experiment for 15 to 20 days. Thus, it can be assumed that the test animals were potassium deficient and alkalotic for at least seven weeks. The control rats were able to maintain a normal carbon dioxide level even though they were presented with the same sodium bicarbonate load as the test rats. This confirms the previous observation that sustained meta-

bolic alkalosis cannot be produced in the rat by the feeding of sodium bicarbonate unless potassium deficiency is present.[2] Also, as has been noted previously,[4,7] the potassium-deficient animals failed to thrive.

The absence of histopathologic changes in the brains of the test animals further demonstrates that the brain is resistant to potassium deficiency. It has been shown that no depletion of potassium occurred in the brains of potassium-deficient rats, although losses were recorded from muscle, heart, and kidney.[4] Lastly, Smith et al.[7] failed to demonstrate central nervous system lesions in three potassium-deficient dogs.

The results of the present experiment may be compared to the work of Windle and co-workers.[9] They did not find any histopathologic changes in the brains of guinea pigs subjected to acute and chronic acidosis. It would appear that the brain is resistant to moderate disturbances in acid-base equilibrium, as demonstrated by present-day histologic techniques.

Summary

Chronic potassium deficiency and alkalosis do not produce any histopathologic changes in the brains of rats.

Drs. Richard G. Berry and Bernard J. Alpers helped in reviewing the histologic sections.

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Peculiar Changes in the Muscles of Patients with Ankylosing Spondylitis

EUGENE F. TRAUT, M.D., and E. W. PASSARELLI, M.D., Chicago

With increased appreciation of the systemic implications of diseases characterized by joint inflammation, changes in tissues other than joints have received increasing attention. The normal and pathological structure and metabolism of mesenchymal tissues are being subjected to especially enthusiastic investigation.

The histology of the soft tissues of rheumatic-fever patients (formerly "acute articular rheumatism") has rightly received more attention than the joints. The Aschoff granuloma is the most persistent characteristic alteration.[1-3]

Structural changes have also been found in the skeletal muscles of patients with rheumatoid arthritis.[4-15]

The position of ankylosing arthritis of the spine (Strümpell-Marie disease, rheumatoid arthritis of the spine, von Bechterew's disease, progressive ankylosing spinal arthritis, spondylitis ankylopoietica, *spondylitis rhizomélique*, spondylitis ossificans ligamentosa, atrophic arthritis of the spine, infectious spondylitis, poker back, spondylarthritis) in the category of rheumatic diseases is not yet settled. It resembles rheumatoid arthritis in its tendency to start in youth—particularly those with an asthenic habitus—in its elevated sedimentation rate, in its histopathological development in the apophyseal joints with a pannus invasion of the synovial membrane, and in its progression to ankylosis. On the other hand, it is unlike rheumatoid arthritis of the extremities in its predilection for the male sex and in its tendency to avoid the smaller peripheral joints. The calcification of adjacent ligamentous structures characterizing ankylosing spondylitis is not seen in rheumatoid arthritis. The symptoms of ankylosing spondylitis are relieved by roentgenologic therapy, as contrasted with the failure of x-ray in peripheral rheumatoid disease. On the other hand, spondylitis resists steroid therapy, contrasting with the usually dramatic response of rheumatoid arthritis.

In an experience of 37 years in a large clinical ward in Cook County Hospital with a changing population of 110 female patients, one of us (E. F. T.) has encountered only three women with typically fused sacroiliac joints and bamboo spines. Two of these also had an extensive peripheral rheumatoid arthritis. In each of these patients the ankylosing arthritis had developed after the climacteric.

An especial tendency for the sedimentation rate to remain elevated was noted in patients with ankylosing spondylitis. It remains elevated steadily through the years, in spite of recession of other signs of active inflammation of the spinal joints. One patient has had no pain for 20 years, and yet, with other causes eliminated, the sedimentation rate remains elevated.

The pain in nerve structures and the atrophy, together with the altered sensitivity and loss of motor function due to nerve

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From the Arthritis Clinic of Cook County Hospital, Chicago, the Presbyterian-St. Luke's Hospital of Chicago and the University of Illinois College of Medicine. Associate Prof. Clinical Medicine (Rush) University of Illinois College of Medicine, Director of Arthritis Clinic at Cook County Hospital, and Associate Attending Physician Presbyterian-St. Luke's Hospital (Dr. Traut); Former Attending Physician to Arthritis Clinic at Cook County Hospital (Dr. Passarelli).

root involvement, have led some, especially early writers, to list ankylosing spondylitis as an entity primarily neurologic and only secondarily skeletal.

The gross and microscopic changes in the bones and joints of patients with ankylosing spondylitis have been extensively described. Changes in the soft tissues, particularly in the muscles, have also received some attention.

Sokoloff, Wilens, Bunim, and McEwen [16] found microscopic changes in the muscles in one-half of their patients with rheumatoid arthritis and in a similar number of patients with Strümpell-Marie disease. They did not note any histopathologic differences between ankylosing arthritis and rheumatoid arthritis of the extremities.

Muscle biopsy specimens from 17 cases of Strümpell-Marie disease studied by Desmaris, Gibson, and Kersley [17] showed none of the round-cell foci and blood vessel changes found in 60% of their 56 patients with rheumatoid arthritis. Hart, Robinson, Allchin, and Maclagan [18] noted no abnormalities in biopsy specimens of muscles from four patients with ankylosing arthritis.

Romanus [19] reported endarteritis and infiltrating cells in the connective tissue structures about the spinal joints involved by ankylosing spondylitis.

Changes in the findings on biopsies of muscle from patients with rheumatoid arthritis in the extremities are characterized by atrophy and degeneration of the muscles, occasionally to the extent of necrosis and scarring. Increase in the number of perimysial nuclei and an infiltration of round cells accompanied the degenerative changes in the fibrils. Accumulations of round cells were seen about the smaller blood vessels.

In connection with a recent study of histologic changes in the muscles of patients with rheumatoid arthritis involving the extremities [20] we also obtained muscle biopsy specimens from patients with ankylosing arthritis of the spine.

Material

Sections of the gastrocnemius muscle were made from 17 men with ankylosing arthritis of the spine. The youngest patient was 28 and the oldest, 60 years of age. Seven patients were less than 45 years old. Eight of them had been completely bedridden from 5 to 40 years in Oak Forest Infirmary or Cook County Hospital. Two patients were ambulatory but unable to work. The disease was very active and associated with severe, constant pain in one patient. The other men were uncomfortable only when making movements involving their backs. All had obliterated sacroiliac joints. Fifteen had calcified spinal ligaments and bamboo spines of varying extent. All of the patients showed severe involvement of the vertebral joints with resulting deformities. All were extremely thin. Their musculature was very sparse and little used.

Inflammatory ("rheumatoid") arthritis with ankylosis also was found in the extremities of 10 of these patients with ankylosing spondylitis.

Two patients had had the symptoms and signs of peripheral rheumatoid arthritis 11 years and 5 years, respectively, before developing pain in the back and x-ray evidence of fused sacroiliac joints. In these two patients x-rays did not demonstrate the calcification of paravertebral ligaments characteristic of the bamboo spine. One had arthritis mutilans with pencil-point distal ends of phalanges fitting into saddle-shaped proximal ends. Many small joints showed subluxation. The proximal joints of the extremities showed bony ankylosis. Both patients had been bed-ridden more than 10 years. The bones of the back and extremities showed severe osteoporosis.

In the other eight spondylitic patients arthritis had more characteristically affected only the proximal limb joints, the hips, and the shoulders, less commonly progressing to the knees and elbows. Three patients had well-compensated mitral stenosis. Another patient with a fixed thorax had marked clubbing of the fingers and toes. No other complications were observed.

The hemoglobin was 12 gm. per 100 cc. or lower (below 9 gm. in four patients). The leukocyte counts varied from 6600 to 12,000, with polymorphonuclear cells relatively increased even in patients with leukocyte counts below 7000. A patient might have a relatively active subacute spondylitis with a daily temperature of 100 F and a sedimentation rate of 40 mm. without any increase in the total number or the relative proportions of the leukocytes. The sedimentation rate varied between 25 and 75 mm. per hour (Westergren technique). In one patient the blood pressure was 148/94; in the others it was 120/76 or lower.

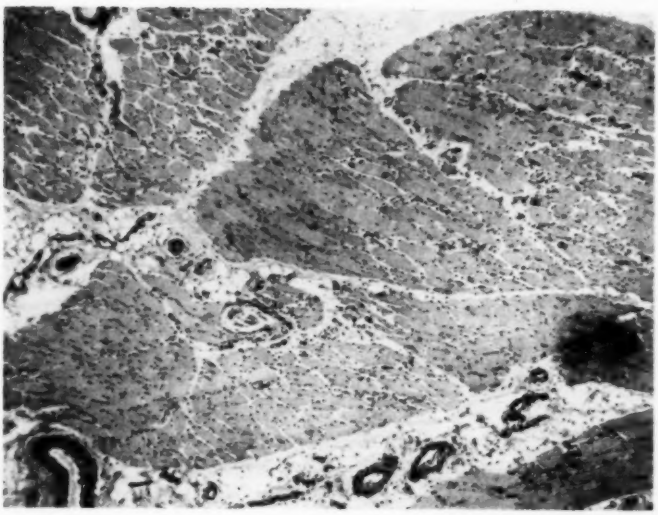

Fig. 1.—Biopsy specimen from gastrocnemius muscle of a patient with ankylosing spondylitis for 15 years. Atrophic fibrils with interfibrillary increase in nuclei. Sensory end-organ. Fat replacement.

Procedure

After infiltration locally with procaine, sections approximately 2 cm. long, 1 cm. wide, and 1 cm. thick were excised from the gastrocnemius muscle about half way between the knee and ankle. Part of the specimen was fixed in formalin; the rest, in Zenker's solution. The sections were stained with hematoxylin and eosin, aniline blue, Mallory's phosphotungstic acid, and silver-lead chloride.

Histopathology

Histopathologically, material from seven patients showed muscle atrophy usually localized and without any inflammatory reaction in, between, or about the muscle fibrils. This focal atrophy affected isolated muscle groups. There was no increase in connective tissue in these atrophic foci but fat replacement did occur. In only one patient was microscopic evidence of muscular atrophy prominent.

In four of these cases there was the periarterial infiltration so regularly encountered in our previously reported patients

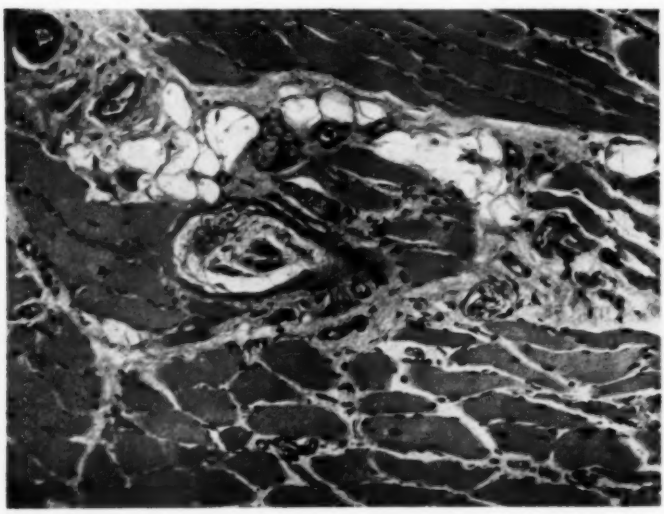

Fig. 2.—Higher magnification of part of Figure 1. End-organ. Fat replacement. Thickened media of arteriole.

Fig. 3.—Section from gastrocnemius muscle of a patient with ankylosing spondylitis for 10 years. Spindle-shaped collagenous structures.

with uncomplicated peripheral rheumatoid arthritis.

In two patients the nerve fibers were large and apparently swollen. The Schwann cells were also seemingly swollen.

Of greatest histopathological interest was the finding of spindle-shaped structures in the muscle sections of six patients. These areas are entirely novel and unprecedented to us and to the pathologists consulted. We had not encountered them in our studies of muscles from patients with rheumatoid arthritis limited to the extremities. The peculiar structures are in the muscle fiber, not about it. The muscle fibrils seem to proceed into these spindle-shaped lesions as they do into a myoma. The muscle fibrils can be traced into these structures and seem to spray out as colorless collagenous threads between the nuclei. They can be regarded as proceeding from focal muscle degeneration and atrophy. The muscle fibril seems

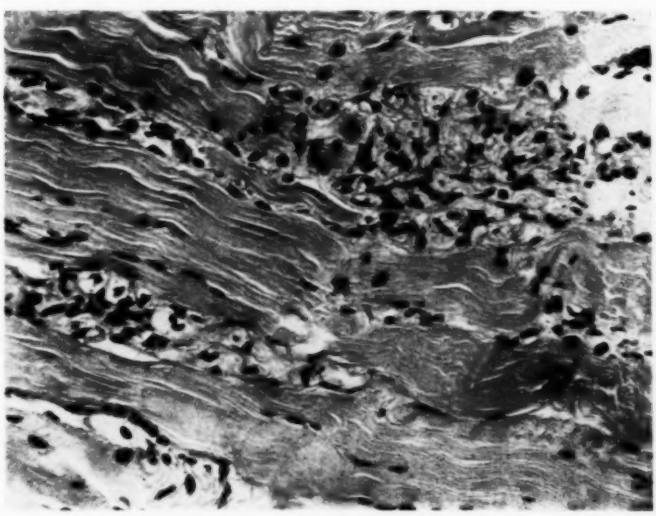

Fig. 4. — More highly magnified section of the biopsy specimen of Figure 3. These intrafibrillary whorls of collagenous fibers are infiltrated with mononuclear cells.

to terminate in a shredded bit of tissue. Mallory's aniline blue stain emphasizes the collagenous nature of these strands into which the muscle fibrils run. The cells of these lesions seem identical with the perimysial cells (Dr. George Hass, Presbyterian-St. Luke's Hospital). There is no order to the arrangement of the nuclei. At first glance they resemble a neuroma similar to those found in amputation stumps.* They do not take the stains for neural tissue (silver stain). The structures appear to be due to proliferation rather than inflammation. They are not muscle spindles. Dr. Eleanor Humphreys and Dr. Richard Richter did not consider them to be endplates. They may be related to nerve endorgans. They are surely neuromuscular. The nerve fibers seem large. The Schwann cells are more prominent due to swelling (Dr. Hans Popper).

Dr. Granville Bennett would not dignify these structures as lesions. He regards them as produced by muscle wasting. According to him the collagenous tissue is only apparently increased due to disappearance of muscle substance. They do not result from inflammation. No structures resembling them were found in preparations of muscle made from a patient with a history of acute anterior poliomyelitis of years previously. The five experienced pathologists consulted regarded the structures as unique.

Summary

Microscopic lesions have been identified in the muscles of patients with ankylosing spondylitis. Such changes have not been seen by us nor reported by others in the muscles of patients with rheumatoid arthritis. These findings are another evidence of the dissimilarity of ankylosing arthritis and rheumatoid arthritis.

Dr. George Hass, of the Presbyterian-St. Luke's Hospital of Chicago; Dr. Granville Bennett, of the University of Illinois; Drs. Richard Richter and Eleanor Humphreys, of The University of Chicago, and Dr. Hans Popper, formerly of the Cook County Hospital assisted in this study.

Drs. Chester Thrift and Kenneth Campione and Eugene Chesrow assisted in obtaining material.

122 S. Michigan Ave.

* Dr. Eleanor Humphreys.

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News and Comment

ANNOUNCEMENTS

Conference on the Chemical Organization of Cells: Normal and Abnormal.—A conference on "The Chemical Organization of Cells: Normal and Abnormal" will be held in Madison, Wis., Aug. 21, 22, and 23, 1958. The following subjects and speakers will be included: "Nutrition and the Cell," G. O. Gey; "Protein Synthesis in the Pituitary," C. H. Li; "The Cell in Development," Paul A. Weiss; "Division of Labor in Cells," G. H. Bourne; "Adaptive Enzymes," Van R. Potter; "Virus and Cell," Frederik B. Bang; "Mechanisms of Hormone Action," C. C. Kochakian; "Nucleoprotein," A. G. Marshak; "Organization of Electron Transport in Cells," David Green; "Pituitary Cell Reactions to Body States," S. C. Sommers; "Organization in Cells of Different Kinds," A. J. Dalton; "Chromosomes, Nucleic Acid, and Information Theory," Henry Quastler.

PERSONAL

Election of Dr. Josiah J. Moore.—Dr. Josiah J. Moore, Chicago, has been elected president of the Institute of Medicine of Chicago. He has also been treasurer of the American Medical Association for the past 15 years.

Award for Dr. May Owen.—Dr. May Owen, of Fort Worth, Texas, recently received the Caldwell Award of the Texas Society of Pathologists.

Books

Gynecological and Obstetric Pathology. By Emil Novak, A.B., M.D., D.Sc. (Hon., Trinity College, Dublin; Tulane) F.A.C.S., F.R.C.O.G. (Hon.), Late Assistant Professor Emeritus of Gynecology, The Johns Hopkins Medical School, and Edmund R. Novak, A.B., M.D., Assistant Professor of Gynecology, The Johns Hopkins Medical School. Price, $14. Pp. 650, with 683 (25 in color) illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1958.

From the time of its appearance as the first comprehensive discussion of obstetric and gynecologic pathology published in the United States, this text by the late Dr. Emil Novak has been recognized as an outstanding work. Through the years it has been polished and the scope somewhat increased in succeeding editions; but the fundamental character has not changed. From the beginning it has been recognized as the personal interpretation of pathologic processes in the female reproductive organs as seen through the eyes of the man who made himself the outstanding figure in this field by looking through a microscope and reading and listening to others also interested in the pathology of the female.

For many years he had looked forward to the continuation of the book by his son when he himself could no longer do it. He told me several years ago it was his most prized possession and the only thing he could leave his son that would be of any value and that he hoped through his son it would continue to be of value to his friends. That his trust was not misplaced is very evident from a perusal of the current edition. The text has been only slightly altered, but a number of new illustrations have been added. The principle changes are in greater elaboration of the clinical and endocrine aspects of pathologic processes and in the addition of a chapter on exfoliative cytopathology by Dr. John K. Frost.

The book is well written, beautifully illustrated, and bound in a manner that makes it a pleasure to handle. Of greatest importance is the fact that of the presentations of gynecologic and obstetric pathology now available it is the most authentic.

The Year Book of Cancer (1956-1957 Year Book Series). Edited by Randolph Lee Clark Jr. and Russell W. Cumley. Price, $7.50. Pp. 572, with 209 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1957.

This new member of the Year Book series contains two hundred seven articles, abstracted by the authors and sometimes followed by editorial comments. Most of the original papers were published during 1955 or in the early months of 1956. This reviewer got the book in March, 1958, so that the lag in some abstracts was over three years. The publisher states that it will be reduced. It is notoriously difficult to review a book of abstracts. The editors write, "The value . . . depends on how judicious has been the selection of articles for abstraction." One can agree with that statement and say that these papers deal with a wide range of subjects but they are of interest chiefly to the clinician.

However, it is possible to go farther and state that the value depends also on the quality of the abstracts, the promptness with which they are published, and the astuteness of the editorial comments. On these scores this book does not fare very well. The quality of the abstracts varies greatly. Many are not abstracts in the true sense; they consist of such voluminous portions as to virtually comprise duplicate publication. For example, a number of the "abstracts" fill five or six pages, and one which was five pages in the original now occupies six and one-half (page 183): The long interval between the publication of the original article and the abstract, which now greatly reduces the value of the latter, will be reduced, it is said. This leaves the editorial work for evaluation. Many abstracts are strung together with no editorial comments. Some editorial comments contradict the article they accompany (e. g., page 281), and one wonders why that paper was selected. Others are inconsistent with the findings presented. Thus the editorial statement is made (page 125), "One should not expect any great advantage to be gained from the use of supervoltage therapy on pulmonary cancer." Nevertheless, a figure is printed on page 135 which shows supervoltage results about as good as from surgery. Ambiguous statements abound such as those on pages 50, 173, 234, 470, 472, and 522; an example is "Carcinogenesis from exogenous materials, such as smoke wastes, is an active field of animal research at present." Finally, the editorial comments introduce some

errors, such as the one on page 417, where the three words, Sir Percival Potts, are all wrong. Editorial liberty sometimes approaches license. For example, neoplasia and malignancy are used for malignant neoplasm. The reviewer is less favorably impressed by this than by any other Year Book he has seen.

Comparative Aspects of Haemolytic Disease of the Newborn. By G. Fulton Roberts, M.A., M.D., Fellow of Jesus College, Cambridge, and Lecturer in Pathology in the University. Price, 17s 6d. Pp. 199. William Heinemann, Ltd., 99 Great Russell St., London, W. C. 1, 1957.

This little book is a review of the current concepts of hemolytic disease, consisting especially of a description of the basic immunologic phenomena concerned in the production of this condition and of the interpretation of their meaning in relation to hemolytic disease as understood by the author. An unusual part of its contents is the inclusion of a discussion of the same condition occurring naturally in horses and pigs and under experimental conditions in dogs, rabbits, mice, and guinea pigs.

It is well written in a narrative prose style that can be easily understood. Anyone interested in the basic considerations of isoimmunization, and especially those concerned with care of the immunized woman or her affected child, cannot help but find the book interesting and of value in understanding the way in which the serologic, clinical, and anatomic changes characteristic of hemolytic disease are produced.

Hemophilia and Hemophilioid Diseases. By Kenneth M. Brinkhous, M.D. Price, $7.50. Pp. 265. University of North Carolina Press, Chapel Hill, N. C., 1957.

This book constitutes the proceedings of an international symposium on hemophilia and hemophilioid diseases sponsored in August, 1956, by the National Hemophilia Foundation.

The views of many of the world's leading authorities on these fascinating diseases are presented under the following headings:

I. The Clotting Defect in Hemophilia
II. The Hemophilioid States
III. Inheritance of Hemophilia and the Hemophilioid States
IV. Diagnosis of Hemophilia
V. Management of Hemorrhage in Hemophilia and Related Diseases
VI. Hemophilic Arthropathy
VII. Psychiatric and Socio-Economic Aspects of Hemophilia

Few other areas of human disease have been studied as intensively or as productively during the last five years. This volume represents a needed compilation of existing opinions discussed in the light of recent developments. Skilfully edited by Dr. Brinkhous, these proceedings should prove stimulating to other workers in medicine, biochemistry, and genetics.

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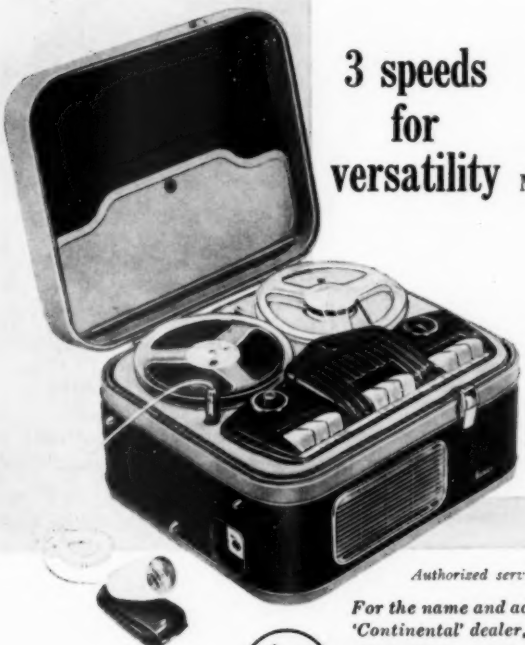

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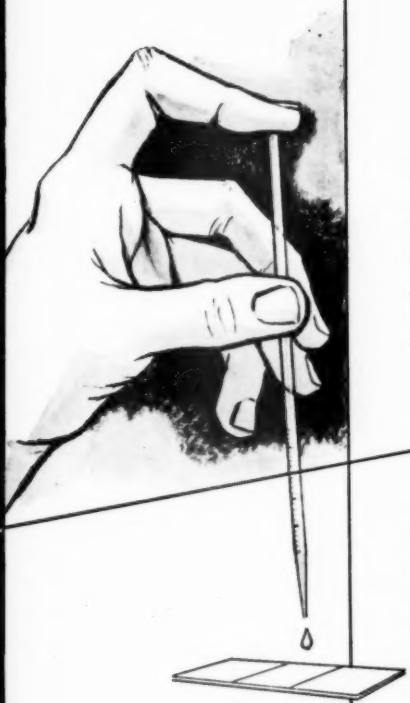

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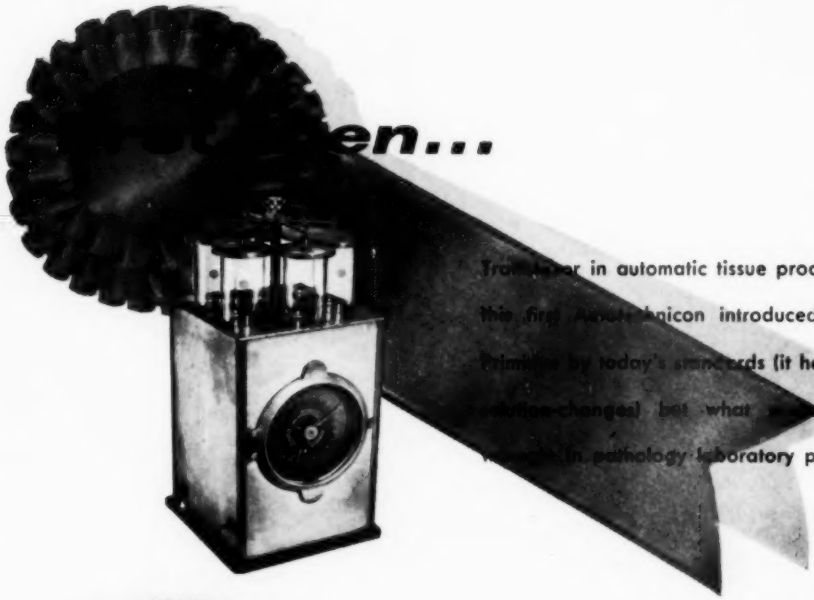

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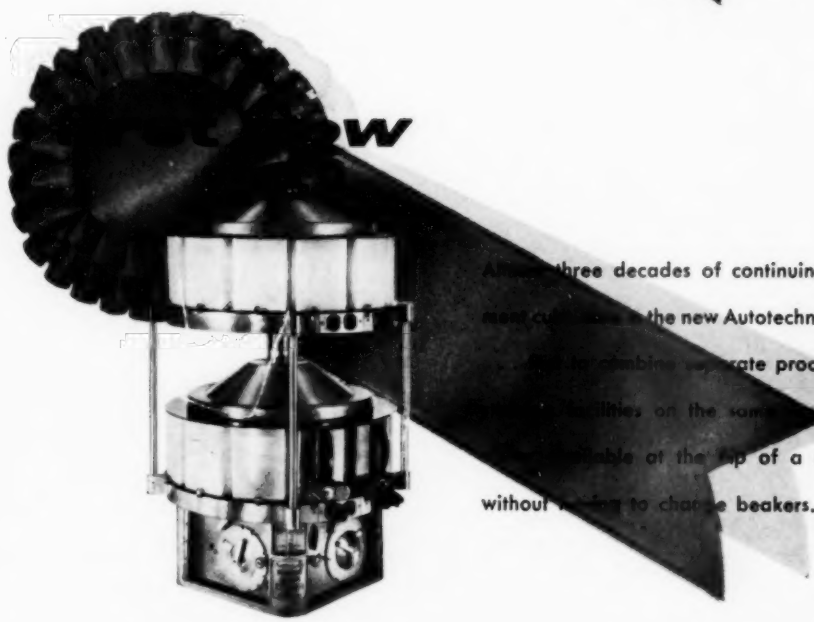

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